Retinoids are not linked to risk of multiple sclerosis
A Danish nationwide cohort study
Cortes-Figueiredo, Filipe; Nielsen, Nete Munk; Stenager, Egon; Paul, Friedemann; Hallas, Jesper; Kristensen, Kasper Bruun

Published in:
European Journal of Neurology

DOI:
10.1111/ene.15116

Publication date:
2022

Document version:
Accepted manuscript

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:

- You may download this work for personal use only.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying this open access version.

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk
Retinoids are not linked to risk of Multiple Sclerosis: a Danish nationwide cohort study

Filipe Cortes-Figueiredo1,2,3*, Nete Munk Nielsen4,5, Egon Stenager6,7, Friedemann Paul2,8, Jesper Hallas3, Kasper Bruun Kristensen3

1 VMorais Lab – Mitochondria Biology & Neurodegeneration, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Portugal
2 NeuroCure Clinical Research Center, Charité - Universitätsmedizin Berlin, Germany
3 Clinical Pharmacology, Pharmacy and Environmental Medicine, Department of Public Health, University of Southern Denmark, Odense, Denmark
4 Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark
5 Focused Research Unit in Neurology, Department of Neurology, Hospital of Southern Jutland, University of Southern Denmark, Denmark
6 MS-clinic of Southern Jutland (Sønderborg, Esbjerg, Kolding), Department of Neurology, Hospital of Southern Jutland, Sønderborg, Denmark
7 Department of Regional Health Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark
8 Experimental and Clinical Research Center, Charité - Universitätsmedizin Berlin and Max Delbrück Center for Molecular Medicine, Germany

* Corresponding author: filipe.figueiredo@edu.ulisboa.pt,
VMorais Lab - Mitochondria Biology & Neurodegeneration, Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

Edificio Egas Moniz, Avenida Professor Egas Moniz, 1649-028 Lisboa

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ENE.15116

This article is protected by copyright. All rights reserved
Total word count: 4988

Keywords: Multiple Sclerosis, Retinoids, Cohort study, Epidemiology, Denmark
Table of contents

I - Abstract (245 words; max: 250) ....................................................................................................................... 2
II - Introduction (282 words) ............................................................................................................................... 2
III – Method (1418 words)..................................................................................................................................... 3
  Study cohort ...................................................................................................................................................... 3
  Drug exposure ................................................................................................................................................... 3
  Identification of MS outcomes ......................................................................................................................... 4
  Covariates and propensity scores .................................................................................................................... 4
  Statistical Analyses ........................................................................................................................................ 5
  Ethical concerns and reporting ...................................................................................................................... 6
IV – Results (498 words).................................................................................................................................... 6
  Figure 1 - Flowchart of the selection of eligible subjects. .................................................................................... 8
  Table 1 - Baseline characteristics of the study cohort before and after three-way propensity score weighting. ................................................................................................................................................. 8
  Figure 2 - Hazard ratios for systemic retinoids compared to non-retinoid acne drugs in subgroups of interest. ....................................................................................................................................................... 8
  Figure 3 - Survival curves for use of non-retinoid acne drugs, topical retinoids and systemic retinoids and incidence of Multiple Sclerosis through follow-up time, i.e., since the first qualifying prescription. ......................................................................................................................................................... 8
  Table 2 - Nested case-control study of cumulative systemic retinoid use in comparison with topical retinoids and non-retinoid acne drugs. ........................................................................................................................................................................ 9
V - Discussion (694 words) ............................................................................................................................... 10
VI - Author Contributions (112 words) ................................................................................................................... 11
VII - Acknowledgments (26 words) ..................................................................................................................... 11
VIII - Funding (34 words) ..................................................................................................................................... 11
IX - Conflicts of Interest (116 words) .................................................................................................................. 12
X – References (1354 words).............................................................................................................................. 12

This article is protected by copyright. All rights reserved
I - Abstract (245 words; max: 250)

Background: Multiple Sclerosis (MS) is a chronic immune-mediated disease of the Central Nervous System with an undetermined etiology. Retinoids may have immunomodulatory effects that favorably influence MS progression. No observational studies have explored the relationship between exposure to retinoids and risk of acquiring MS.

Methods: We performed a nationwide cohort study in the Danish population in the period 1998-2016 comparing MS incidence in three groups: users of systemic retinoids, users of topical retinoids (negative control group) and users of non-retinoid acne drugs (control group). We used data from the Danish Multiple Sclerosis Registry (DMSR), the Danish National Prescription Registry and The Danish National Patient Registry. Linkage was obtained through the personal identification number (CPR number). We addressed confounding by three-way propensity score (PS) matching weights. Additionally, to evaluate a cumulative dose-response effect for systemic retinoids on MS incidence, we conducted a case-control study nested within the cohort.

Results: A total of 257,193 users of non-retinoid acne drugs, 130,560 users of topical retinoids, and 75,610 users of systemic retinoids were included. Systemic retinoid use was not associated with a reduced risk of MS compared to non-retinoid acne drugs in crude (HR 0.80 [95% CI, 0.61 to 1.05]) and weighted analyses (HR 0.89 [95% CI, 0.67 to 1.20]). There was no evidence of a cumulative dose-response association between systemic retinoids and MS incidence.

Conclusions: Use of systemic retinoids was not associated with a reduced incidence of MS compared to use of non-retinoid acne drugs in this study.

II - Introduction (282 words)

Multiple Sclerosis (MS) is an immune-mediated disease that causes demyelination and neuroaxonal loss in the Central Nervous System with a heterogeneous and potentially debilitating clinical course. With a still unknown etiology, the cause is believed to be multifactorial, with genetic predisposition and environmental factors both playing a role.

Retinoids (vitamin A analogues) have been extensively researched in mice with experimental autoimmune encephalomyelitis (EAE), an animal model of MS. These studies have shown that exposure to retinoids causes an immunosuppression through a decrease in antigen-presenting cells, a repression of T helper (Th) 17 cells, a repression of the production of interleukin (IL)-17 by γδ T cells and through an increase of Treg cells. Retinoid treatment in these animals has also led to remyelination and a decrease in disease morbidity.

Although vitamin A levels, in general, are normal in patients with MS, clinical studies have suggested that lower serum retinol levels might result in higher imaging disease burden, and in vitro studies suggest that exposure to retinoids has an immunomodulatory effect. Furthermore, one double-blind randomized clinical trial with RRMS patients showed that a 1-year treatment with retinyl palmitate caused a significant improvement of the Multiple Sclerosis Functional Composite (MSFC) score, even though relapse rates, expanded disability This article is protected by copyright. All rights reserved
status scale (EDSS), and T2 and Gadolinium-enhancing lesions did not significantly change.\textsuperscript{17} Finally, an ongoing trial is exploring the remyelination potential of a retinoid treatment with bexarotene in patients with relapsing-remitting MS.\textsuperscript{18}

No observational studies have explored the relationship between exposure to retinoids and MS incidence. Retinoids are a mainstay of acne treatment,\textsuperscript{19,20} thus offering a unique opportunity to study the association between systemic retinoid exposure and subsequent MS diagnoses in an observational study.

\textbf{III – Method (1418 words)}

\textit{Study cohort}

Using information from the Danish National Prescription Registry,\textsuperscript{21} we identified three cohorts of Danish individuals initiating therapy with systemic retinoids, topical retinoids and non-retinoid drugs used to treat acne between 1\textsuperscript{st} January 1998 and 31\textsuperscript{st} December 2016. Those who had received any of these treatments before the start of the study period were excluded to ensure participation of only new users. Further, all eligible individuals were required to have resided continuously in Denmark 12 months prior to cohort entry, to be aged 10 years or above at cohort entry, and not have a diagnosis of MS prior to cohort entry. The above individuals were identified from a source population that consisted of all Danish residents born between 1\textsuperscript{st} January 1975 to 31\textsuperscript{st} December 1999.

\textit{Drug exposure}

Drug use was retrieved from the Danish National Prescription Registry, established in 1995.\textsuperscript{21} This registry contains individual-level information on all prescriptions filled at all Danish pharmacies and each record includes the personal identification number, dispensing date, anatomic therapeutic chemical (ATC) code,\textsuperscript{22} and number of defined daily doses.

We used a hierarchical design using three different exposure levels:

- Systemic retinoid cohort, with individuals filling a prescription for oral isotretinoin (ATC code D10BA);
- Topical retinoid cohort, with individuals filling a prescription for topical retinoids (ATC code D10AD);
- Non-retinoid cohort, with individuals filling a prescription for non-retinoid acne medications, i.e., non-retinoid topical drugs (ATC codes D10AA, D10AB, D10AE, D10AF, and D10AX) and contraceptives with an antiandrogenic effect (ATC code G03HB01).

We included topical retinoids as separate cohort serving as a negative control, since bioavailability following application is negligible\textsuperscript{23,24} and since the similarity between systemic and topical retinoid users would eliminate some potential confounding.

This article is protected by copyright. All rights reserved
In clinical practice, patients switch between anti-acne regimes, and accordingly, the analysis should allow for change in exposure status. We adopted a hierarchical exposure classification where individuals were allowed to move from the non-retinoid cohort to the topical retinoid cohort to the systemic retinoid cohort, i.e., upward in the hierarchy. However, switches downward in the hierarchy were disregard, e.g., from systemic retinoids to topical retinoids. This procedure was chosen to reflect a belief that a possible effect of systemic retinoids on MS would be cumulative rather than an effect of ongoing treatment and that it would linger after discontinuation. Individuals who switched cohort were censored from the original cohort and began contributing person-time to the new cohort at the time of the prescription fill for the new drug. This means that the same individual would be included in more than one cohort if that individual switched treatment upward in the hierarchy during the study.

Identification of MS outcomes

Incident MS outcomes were identified using the Danish Multiple Sclerosis Registry (DMSR), where notifications of possible MS cases have been reviewed, validated, and classified by neurologists, with very high validity and completeness. Cases were classified according to the Poser criteria from 1994 and the McDonald Criteria from 2005. The date of MS onset was defined as the debut date, i.e., date of first symptoms. In most cases, the debut date is recorded according to calendar year in the DMSR which was operationally defined as 1st July. To ensure that the outcome did not precede the exposure, we excluded individuals if the calendar year of MS onset coincided with the calendar year of cohort entry.

Covariates and propensity scores

In order to adjust for baseline differences at cohort entry in the probability of receiving retinoid treatment, we estimated propensity scores (PS) for each comparison group, i.e., systemic retinoids, topical retinoids and non-retinoid acne drugs. In each PS model, we included the following potential confounders: sex, age, highest achieved education, ever occurrence of selected hospital diagnoses (including disorders with high prevalence and morbidity in the Danish population) and, finally, drug use within 365 days before cohort entry.

Highest achieved education was obtained from Statistics Denmark, and education levels were classified according to the national classification of educational programs. Information about morbidities was obtained from the National Patient Registry. We obtained information on primary and secondary diagnoses from all hospitalizations, outpatient visits, and emergency department visits in Denmark since January 1994. Diagnoses were classified according to the WHO international classification of diseases, version 10 (ICD-10). Finally, information on filled drug prescriptions at community pharmacies were obtained from the Danish National Prescription Registry. ICD-10 codes and ATC codes included in the PS estimation are listed in Table S1.

As tetracyclines are widely used in acne treatment, we initially included them as potential confounders in our PS model. However, after a preliminary analysis showed they were mostly associated with exposure rather than outcome, we considered them as an instrumental variable and, thus, omitted them.

This article is protected by copyright. All rights reserved
Statistical Analyses

Subjects were followed from their first qualifying prescription until the earliest of the following: death, emigration, switch to a drug upwards in hierarchy, onset of the outcome or end of the study period (31-12-2016). Patients were censored at the competing events: switching between medication upward in the hierarchy, death, and migration. Thus, competing events were handled using the cause-specific approach.

We used an intention to treat approach within the hierarchical exposure classification. That is, for each cohort, an individual was considered exposed from cohort entry until censoring (e.g., switch upwards in hierarchy) or an outcome event.

We estimated propensity scores for initiating systemic retinoids, topical retinoids, and non-retinoid acne drugs, i.e., for each subject, three PS were estimated using multinomial logistic regression. The variables included in the PS models were all the ones mentioned under the covariates section (Table S1). The variables were measured at entry into a given exposure level and were thus allowed to differ for the same individual if that individual switched exposure during the study. Propensity scores were estimated separately in six strata according to calendar time of cohort entry (1998-2001, 2002-2004, 2005-2007, 2008-2010, 2011-2013, 2014-2016) to avoid assumptions about stable utilization patterns and physician preferences over time, i.e., a person could have the PS estimated several times if contributing to more than one cohort. We used three-way matching weights based on PS to account for confounding. This method is well suited for settings with more than two exposure groups and rare outcomes. The matching weights were calculated as the smallest value of the three estimated propensity scores divided by the propensity score of the treatment actually received. The estimand of this weighting method is asymptotically equal to exact three-way PS matching.

The incidence of MS in the three groups was visualized in cumulative incidence curves using the Kaplan Meier technique. We estimated incidence rates (IR) and incidence rate differences (IRD) with 95% confidence intervals in univariate analyses based on Poisson models. Hazard ratios (HR) were estimated using univariate Cox proportional hazards regression. IRD and HR were calculated for topical retinoids compared to non-retinoid acne drugs, and systemic retinoids compared to non-retinoid acne drugs. These analyses were conducted as crude and weighted analyses. Since each individual could contribute to more than one exposure category, we calculated clustered robust variance estimates. The proportional hazard assumption was checked using Schoenfeld residuals. Differences in characteristics between the three exposure groups were described using standardized mean differences (SMD). The following subgroup analyses were also performed: stratifying on sex (male vs. female) and age at cohort entry (10-15, 16-20, >20 years).

Finally, in order to evaluate cumulative dose-response effects, we conducted a case-control study nested in the three cohorts. Controls were selected by risk-set sampling, i.e., to each case, we matched 20 controls on time since cohort entry (i.e., follow-up duration), birthyear, and sex using risk-set sampling. Cases were eligible as controls before they became cases. Controls were assigned an index date corresponding to the date of MS onset for the case they were matched to. We collected information on use of non-retinoid acne drugs, topical retinoids and systemic retinoids for cases and controls before the index date. Systemic retinoid use was categorized according to the cumulative dose expressed as defined daily doses (DDDs). Odds ratios were calculated using
conditional logistic regression with no use of systemic retinoid (i.e., only use of non-retinoid acne drugs or topical retinoids) as reference.

**Ethical concerns and reporting**

Registry-based studies do not require patient consent or approval from an ethics review board according to Danish law. Individual-level data cannot be shared by the authors for legal reasons. Linkage was obtained through the personal identification number (CPR number). Approval was obtained by the Danish Data Protection Agency (18/30233), the DMSR (on September 30, 2017) and Statistics Denmark (707136). Reporting followed the standards from STROBE, RECORD, and RECORD-PE.

IV – Results (498 words)

We identified 268,434 new users of non-retinoid acne drugs, 134,881 new users of topical retinoids, and 77,082 new users of systemic retinoids, of whom 257,193, 130,560, and 75,610 were eligible for inclusion, respectively (Figure 1 and Figure S1). Baseline characteristics are shown in Table 1.

Compared to users of non-retinoid acne drugs and of topical retinoids, users of systemic retinoids were slightly older, predominantly male, and more likely to be diagnosed with hospital-diagnosed acne (Table 1).

After PS weighting (Figure S2), the SMD was less than 0.1 for all baseline covariates, indicating successful balancing of measured confounders and covariates (Table 1). Despite the crude MS incidence rate (IR) per 100,000 person-years being slightly higher in users of non-retinoid acne drugs compared to users of topical and systemic retinoid drugs, the HR were statistically similar between the three groups (Figure 2 and Table S2). Following PS weighting, the IR was 13.1 [95% CI, 11.3 to 15.3] for non-retinoid users, 12.5 [95% CI, 10.2 to 15.4] for topical retinoid users, and 12.0 [95% CI, 9.4 to 15.6] for systemic retinoid users. The HR comparing topical retinoid users to non-retinoid acne drug users was 0.95 [95% CI, 0.73 to 1.22] and the HR comparing systemic retinoid users to non-retinoid acne drug users was 0.89 [95% CI, 0.67 to 1.20]. Subgroup analyses for sex showed no significant differences between men and women. Adolescents (treatment start at ages 16-20 years) showed a crude reduced HR for systemic retinoids although the same was not observed after weighting (Figure 2 and Table S2). The Kaplan-Meier curves are shown in Figure 3.

Additionally, taking into account a likely prodromal period between cohort entry and MS diagnosis, we conducted a sensitivity analysis where the first 5 years after exposure were not included as time-at-risk. This yielded a weighted HR of 0.89 [95% CI, 0.59 to 1.33] for systemic retinoids in comparison to non-retinoid acne drugs.

In the nested case-control study we did not observe a dose-response association between increasing doses of systemic retinoids compared to use of topical retinoids and/or non-retinoid acne drugs (Table 2).

This article is protected by copyright. All rights reserved
FIGURE 1

Figure 1 - Flowchart of the selection of eligible subjects.
Abbreviations: MS, Multiple Sclerosis.

Table 1 - Baseline characteristics of the study cohort before and after three-way propensity score weighting.

TABLE 1
† Individuals may contribute to more than one cohort
Abbreviations: excl., excluding; IQR, interquartile range; SMD, standardized mean difference.

FIGURE 2

Figure 2 - Hazard ratios for systemic retinoids compared to non-retinoid acne drugs in subgroups of interest.
Abbreviations: CI, confidence interval; HR, hazard ratio.

FIGURE 3

Figure 3 - Survival curves for use of non-retinoid acne drugs, topical retinoids and systemic retinoids and incidence of Multiple Sclerosis through follow-up time, i.e., since the first qualifying prescription.
Abbreviations: MS, Multiple Sclerosis; n, number.

This article is protected by copyright. All rights reserved
Table 2 - Nested case-control study of cumulative systemic retinoid use in comparison with topical retinoids and non-retinoid acne drugs.

TABLE 2

Abbreviations: CI, confidence interval; DDDs, defined daily dose; OR, odds ratio; ref, reference.
We performed a nationwide cohort study to investigate the relationship between exposure to retinoids and the risk of being diagnosed with MS.

We observed no association between use of retinoids and incidence of MS, either for the whole cohort or within specific sex and age groups. We also looked into cumulative doses of systemic retinoids in a nested case-control study, and found no evidence of a dose-response effect between exposure to systemic retinoids and MS risk. These results are not fully in line with previous clinical trials and experimental studies, where retinoids exerted immunomodulatory effects that favorably influenced MS progression, while potentially promoting remyelination.

One important distinction between these studies and ours is that our study addresses MS incidence and not disease activity or progression, which could potentially have entirely different underlying biological mechanisms. A notable example is Epstein-Barr virus (EBV) infection, which, despite being a highly credible risk factor for MS onset, remains controversial as a predictor of disease progression.

This is the first observational study exploring the relationship between systemic exposure to retinoids and incidence of MS. Our study has several strengths. We identified all exposures to non-retinoid acne drugs, topical retinoids and systemic retinoids through a national prescription registry and have used nationwide data from validated, reliable and continuously updated registries for both the outcome and the covariates, which greatly reduces selection bias, as well as recall bias. Additionally, by using an active comparator - topical retinoids - as a negative control, we are able to reduce the impact of unmeasured confounding.

Our study also has a number of limitations. Firstly, we cannot exclude residual confounding. Since environmental risk factors and protective factors for MS remain largely unknown, it is difficult to assess the degree of this potential bias. Secondly, it was difficult to ascertain the precise onset of the disease, similarly to many other insidious and chronic diseases, which could potentially have introduced a non-differential misclassification. However, the sensitivity analysis we conducted excluding the first 5 years after exposure as time-at-risk still yielded a null HR. Thirdly, we considered all filled prescriptions as definite exposures when, in fact, 58% of patients treated for acne in Europe, including Denmark, have shown poor treatment adherence. Nonetheless, our cumulative dose-response analysis gave a null result as well. Those in the high dose categories have redeemed multiple prescriptions and are unlikely to be consistent non-users. Fourth, our study cohort mainly represents people treated for acne, having an overrepresentation of males for systemic retinoid users, and a substantially younger age in the three cohorts, in comparison with data from the overall Danish population for the past decade. Thus, our study is not representative for the general population at risk of MS. A final limitation is the somewhat limited statistical precision. Our main estimate was a HR of 0.80, albeit with a confidence interval overlapping the null value. We cannot rule out that a moderate protective effect has been overlooked. However, given this is a study on incidence of MS, the clinical correlate would be to use such retinoids to prevent MS in high-risk patients. It is doubtful that a preventive effect of this magnitude would find widespread clinical utility.

The study population reflects the epidemiology of acne, which usually arises between the ages of 14 and 16 years, affecting a majority of adolescents and decreasing with age. The overrepresentation of females in non-This article is protected by copyright. All rights reserved
retinoid acne drug users and topical retinoid users is also consistent with a majority of female acne patients in clinical practice,\textsuperscript{44,47,48} despite a male majority in adolescence.\textsuperscript{47} As oral isotretinoin is usually a second-line treatment for more severe forms of acne,\textsuperscript{19,20} the male predominance in systemic retinoid users might arise from either the teratogenicity of isotretinoin\textsuperscript{20,50} or from an increased extension of acne lesions and/or a higher prevalence of scars in male patients.\textsuperscript{44,49} The slightly older age of systemic retinoid users might also be explained by its use as a second-line option, which is consistent with the older age at the start of oral treatments observed in clinical practice.\textsuperscript{44}

In summary, use of retinoids was not associated with a reduced incidence of MS in this nationwide cohort study.

VI - Author Contributions (112 words)

Filipe Cortes-Figueiredo: Conceptualization (lead); Methodology (equal); Supervision (supporting); Writing – Original Draft Preparation (lead); Writing – Review & Editing (equal).

Nete Munk Nielsen: Conceptualization (supporting); Methodology (equal); Supervision (supporting); Writing – Original Draft Preparation (supporting); Writing – Review & Editing (equal).

Egon Stenager: Conceptualization (supporting); Supervision (supporting); Writing – Original Draft Preparation (supporting); Writing – Review & Editing (equal).

Friedemann Paul: Funding Acquisition (equal); Writing – Original Draft Preparation (supporting); Writing – Review & Editing (equal).

Jesper Hallas: Conceptualization (supporting); Formal Analysis (supporting); Funding Acquisition (equal); Methodology (equal); Supervision (lead); Writing – Original Draft Preparation (supporting); Writing – Review & Editing (equal).

Kasper Bruun Kristensen: Formal Analysis (lead); Methodology (equal); Supervision (supporting); Writing – Original Draft Preparation (supporting); Writing – Review & Editing (equal).

VII - Acknowledgments (26 words)

We would like to thank Melinda Magyari and Bjarne Laursen for their support in obtaining data from the DMSR and Martin Thomsen for independent code validation.

VIII - Funding (34 words)

FCF’s stipend was paid by Fundação para a Ciência e Tecnologia (FCT) – reference PD/BD/114122/2015, and KBK was funded by the Independent Research Fund Denmark and the Research Fund of the Region of Southern Denmark.
IX - Conflicts of Interest (116 words)

Filipe Cortes-Figueiredo: Nothing to disclose.
Nete Munk Nielsen: Nothing to disclose.
Egon Stenager: Nothing to disclose.

Friedemann Paul: FP has received honoraria from advisory boards, travel support, speaking fees and/or financial support for research activities from Bayer, Novartis, Biogen, Teva, Sanofi-Aventis/Genzyme, Merck Serono, Alexion, Chugai, MedImmune, Shire, German Research Council, Werth Stiftung of the City of Cologne, German Ministry of Education and Research, EU FP7 Framework Program, Arthur Arnstein Foundation Berlin, Guthy Jackson Charitable Foundation, and National Multiple Sclerosis of the USA. He serves as academic editor for PLoS ONE and associate editor for Neurology, Neuroimmunology and Neuroinflammation. None resulted in a conflict of interest.

Jesper Hallas: Nothing to disclose.
Kasper Bruun Kristensen: Nothing to disclose.

X – References (1354 words)
Research data are not shared. According to Danish law, data cannot be shared with third parties.


This article is protected by copyright. All rights reserved.


This article is protected by copyright. All rights reserved


This article is protected by copyright. All rights reserved


This article is protected by copyright. All rights reserved


This article is protected by copyright. All rights reserved
### Crude

<table>
<thead>
<tr>
<th>Users of non-retinoid acne drugs (N = 257,193)</th>
<th>Users of topical retinoids (N = 130,560)</th>
<th>Users of systemic retinoids (N = 75,610)</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>17 (15-22)</td>
<td>18 (16-22)</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>Male sex</td>
<td>96,626</td>
<td>37.6</td>
<td>53,613</td>
<td>41.1</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital-diagnosed acne</td>
<td>346</td>
<td>0.1</td>
<td>541</td>
<td>0.4</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>443</td>
<td>0.2</td>
<td>204</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A or D disturbances</td>
<td>250</td>
<td>0.1</td>
<td>168</td>
<td>0.1</td>
</tr>
<tr>
<td>Pro-inflammatory &amp; pro-comedogenic disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic ovarian syndrome</td>
<td>913</td>
<td>0.4</td>
<td>292</td>
<td>0.2</td>
</tr>
<tr>
<td>Hospital-diagnosed obesity</td>
<td>3,717</td>
<td>1.4</td>
<td>1,386</td>
<td>1.1</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>30</td>
<td>0.0</td>
<td>28</td>
<td>0.0</td>
</tr>
</tbody>
</table>

### Weighted

<table>
<thead>
<tr>
<th>Users of non-retinoid acne drugs (N = 72,788)</th>
<th>Users of topical retinoids (N = 73,032)</th>
<th>Users of systemic retinoids (N = 73,236)</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>18 (15-22)</td>
<td>18 (16-22)</td>
<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Male sex</td>
<td>40,180</td>
<td>55.2</td>
<td>40,418</td>
<td>55.3</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital-diagnosed acne</td>
<td>324</td>
<td>0.4</td>
<td>325</td>
<td>0.4</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>132</td>
<td>0.2</td>
<td>131</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A or D disturbances</td>
<td>70</td>
<td>0.1</td>
<td>69</td>
<td>0.1</td>
</tr>
<tr>
<td>Pro-inflammatory &amp; pro-comedogenic disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polycystic ovarian syndrome</td>
<td>185</td>
<td>0.3</td>
<td>187</td>
<td>0.3</td>
</tr>
<tr>
<td>Hospital-diagnosed obesity</td>
<td>777</td>
<td>1.1</td>
<td>769</td>
<td>1.1</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>9</td>
<td>0.0</td>
<td>10</td>
<td>0.0</td>
</tr>
</tbody>
</table>

This article is protected by copyright. All rights reserved
<table>
<thead>
<tr>
<th>Condition</th>
<th>Crude Users of non-retinoid acne drugs ($N = 257,193$)</th>
<th>Users of topical retinoids ($N = 130,560$)</th>
<th>Users of systemic retinoids ($N = 75,610$)†</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
<th>Weighted Users of non-retinoid acne drugs ($N = 72,788$)</th>
<th>Users of topical retinoids ($N = 73,032$)</th>
<th>Users of systemic retinoids ($N = 73,236$)†</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyslipidemia</td>
<td>186 (0.1)</td>
<td>88 (0.1)</td>
<td>43 (0.1)</td>
<td>0.00</td>
<td>0.01</td>
<td>40 (0.1)</td>
<td>41 (0.1)</td>
<td>41 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cancer</td>
<td>631 (0.2)</td>
<td>343 (0.3)</td>
<td>192 (0.3)</td>
<td>0.00</td>
<td>0.00</td>
<td>180 (0.2)</td>
<td>176 (0.2)</td>
<td>182 (0.2)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Diseases of blood and blood-forming organs</td>
<td>769 (0.3)</td>
<td>440 (0.3)</td>
<td>221 (0.3)</td>
<td>0.01</td>
<td>0.00</td>
<td>214 (0.3)</td>
<td>214 (0.3)</td>
<td>213 (0.3)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Immune-mediated disorders</td>
<td>5,276 (2.1)</td>
<td>2,796 (2.1)</td>
<td>1,487 (2.0)</td>
<td>0.01</td>
<td>0.01</td>
<td>1,417 (1.9)</td>
<td>1,413 (1.9)</td>
<td>1,421 (1.9)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cardiovascular diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>154 (0.1)</td>
<td>69 (0.1)</td>
<td>39 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
<td>37 (0.1)</td>
<td>37 (0.1)</td>
<td>38 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>344 (0.1)</td>
<td>181 (0.1)</td>
<td>100 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
<td>91 (0.1)</td>
<td>90 (0.1)</td>
<td>91 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Lower respiratory diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower respiratory tract infections</td>
<td>10,330 (4.0)</td>
<td>6,317 (4.8)</td>
<td>3,604 (4.8)</td>
<td>0.04</td>
<td>0.04</td>
<td>3,511 (4.8)</td>
<td>3,532 (4.8)</td>
<td>3,527 (4.8)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

This article is protected by copyright. All rights reserved.
<table>
<thead>
<tr>
<th>Condition</th>
<th>Users of non-retinoid acne drugs (N = 257,193)</th>
<th>Users of topical retinoids (N = 130,560)</th>
<th>Users of systemic retinoids (N = 75,610)</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
<th>Users of non-retinoid acne drugs (N = 72,788)</th>
<th>Users of topical retinoids (N = 73,032)</th>
<th>Users of systemic retinoids (N = 73,236)†</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic lung disease</td>
<td>12,178 (4.7)</td>
<td>6,872 (5.3)</td>
<td>4,111 (5.4)</td>
<td>0.02</td>
<td>0.03</td>
<td>3,964 (5.4)</td>
<td>3,989 (5.5)</td>
<td>3,992 (5.5)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Migraine</td>
<td>2,200 (0.9)</td>
<td>1,263 (1.0)</td>
<td>791 (1.0)</td>
<td>0.01</td>
<td>0.02</td>
<td>758 (1.0)</td>
<td>749 (1.0)</td>
<td>759 (1.0)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1,129 (0.4)</td>
<td>504 (0.4)</td>
<td>283 (0.4)</td>
<td>0.01</td>
<td>0.01</td>
<td>269 (0.4)</td>
<td>267 (0.4)</td>
<td>269 (0.4)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pregnancy, childbirth, and puerperium</td>
<td>22,875 (8.9)</td>
<td>10,038 (7.7)</td>
<td>5,078 (6.7)</td>
<td>0.04</td>
<td>0.08</td>
<td>5,057 (6.9)</td>
<td>5,048 (6.9)</td>
<td>5,033 (6.9)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td><strong>Prescription drug use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics, dermatologic</td>
<td>10,386 (4.0)</td>
<td>4,933 (3.8)</td>
<td>2,417 (3.2)</td>
<td>0.01</td>
<td>0.05</td>
<td>2,350 (3.2)</td>
<td>2,355 (3.2)</td>
<td>2,352 (3.2)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>preparations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A or D</td>
<td>129 (0.1)</td>
<td>75 (0.1)</td>
<td>34 (0.0)</td>
<td>0.00</td>
<td>0.00</td>
<td>29 (0.0)</td>
<td>32 (0.0)</td>
<td>30 (0.0)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Drugs used for smoking cessation</td>
<td>217 (0.1)</td>
<td>111 (0.1)</td>
<td>74 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
<td>65 (0.1)</td>
<td>64 (0.1)</td>
<td>66 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Drugs used for weight loss</td>
<td>1,256 (0.5)</td>
<td>375 (0.3)</td>
<td>171 (0.2)</td>
<td>0.03</td>
<td>0.04</td>
<td>169 (0.2)</td>
<td>167 (0.2)</td>
<td>166 (0.2)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Lipid lowering drugs</td>
<td>332 (0.1)</td>
<td>141 (0.1)</td>
<td>80 (0.1)</td>
<td>0.01</td>
<td>0.01</td>
<td>76 (0.1)</td>
<td>71 (0.1)</td>
<td>74 (0.1)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

This article is protected by copyright. All rights reserved.
### Crude

<table>
<thead>
<tr>
<th>Drugs used for cancer and immune disorders</th>
<th>Users of non-retinoid acne drugs ($N = 257,193$)</th>
<th>Users of topical retinoids ($N = 130,560$)</th>
<th>Users of systemic retinoids ($N = 75,610$)$^\dagger$</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------------------------------</td>
<td>------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Drugs used for cancer and immune disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal anti-inflammatory drugs</td>
<td>778 0.3</td>
<td>447 0.3</td>
<td>194 0.3</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Corticosteroids for systemic use</td>
<td>3,563 1.4</td>
<td>1,702 1.3</td>
<td>1,151 1.5</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Antineoplastic and immunomodulating drugs</td>
<td>869 0.3</td>
<td>431 0.3</td>
<td>188 0.2</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>Local immunosuppressants</td>
<td>6,257 2.4</td>
<td>2,881 2.2</td>
<td>1,887 2.5</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Chemotherapeutics for topical use</td>
<td>28,482 11.1</td>
<td>13,926 10.7</td>
<td>7,634 10.1</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Corticosteroids, dermatological preparations</td>
<td>1,207 0.5</td>
<td>668 0.5</td>
<td>439 0.6</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

### Weighted

<table>
<thead>
<tr>
<th>Drugs used for cancer and immune disorders</th>
<th>Users of non-retinoid acne drugs ($N = 72,788$)</th>
<th>Users of topical retinoids ($N = 73,032$)</th>
<th>Users of systemic retinoids ($N = 73,236$)$^\dagger$</th>
<th>SMD, topical vs non-retinoids</th>
<th>SMD, systemic vs non-retinoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------------------------------</td>
<td>------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Drugs used for cancer and immune disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal anti-inflammatory drugs</td>
<td>181 0.2</td>
<td>181 0.2</td>
<td>180 0.2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Corticosteroids for systemic use</td>
<td>1,051 1.4</td>
<td>1,040 1.4</td>
<td>1,055 1.4</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Antineoplastic and immunomodulating drugs</td>
<td>181 0.2</td>
<td>169 0.2</td>
<td>175 0.2</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Local immunosuppressants</td>
<td>1,748 2.4</td>
<td>1,732 2.4</td>
<td>1,754 2.4</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Chemotherapeutics for topical use</td>
<td>7,323 10.1</td>
<td>7,220 9.9</td>
<td>7,334 10.0</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Corticosteroids, dermatological preparations</td>
<td>404 0.6</td>
<td>393 0.5</td>
<td>400 0.5</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>404 0.6</td>
<td>393 0.5</td>
<td>400 0.5</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

This article is protected by copyright. All rights reserved.
<table>
<thead>
<tr>
<th>Preparations, excl. corticosteroids</th>
<th>Crude</th>
<th>Weighted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antipsoriatic drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>762</td>
<td>241</td>
</tr>
<tr>
<td>%</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Antiallergics</td>
<td>395</td>
<td>230</td>
</tr>
<tr>
<td>%</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Nasal corticosteroids</td>
<td>1,151</td>
<td>3,329</td>
</tr>
<tr>
<td>%</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Inhalation glucocorticoids alone or in combination with adrenergics</td>
<td>4,407</td>
<td>2,115</td>
</tr>
<tr>
<td>%</td>
<td>3.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Antimigraine drugs</td>
<td>1,382</td>
<td>2,142</td>
</tr>
<tr>
<td>%</td>
<td>1.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Antidiabetic drugs</td>
<td>621</td>
<td>2,139</td>
</tr>
<tr>
<td>%</td>
<td>0.5</td>
<td>2.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None or basic education</td>
<td>185,059</td>
<td>50,215</td>
</tr>
<tr>
<td>%</td>
<td>72.0</td>
<td>69.0</td>
</tr>
<tr>
<td>High school or vocational training</td>
<td>63,582</td>
<td>19,765</td>
</tr>
<tr>
<td>%</td>
<td>24.7</td>
<td>27.2</td>
</tr>
<tr>
<td>Higher education</td>
<td>8,552</td>
<td>2,809</td>
</tr>
<tr>
<td>%</td>
<td>3.3</td>
<td>3.9</td>
</tr>
</tbody>
</table>

This article is protected by copyright. All rights reserved
<table>
<thead>
<tr>
<th>Cumulative consumption of systemic retinoids</th>
<th>Controls</th>
<th>Cases</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 DDDs</td>
<td>7,503</td>
<td>374</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>1 – 150 DDDs</td>
<td>260</td>
<td>10</td>
<td>0.77 (0.41 to 1.46)</td>
</tr>
<tr>
<td>151 – 250 DDDs</td>
<td>382</td>
<td>24</td>
<td>1.26 (0.82 to 1.93)</td>
</tr>
<tr>
<td>251 – 350 DDDs</td>
<td>329</td>
<td>15</td>
<td>0.91 (0.54 to 1.56)</td>
</tr>
<tr>
<td>&gt;350 DDDs</td>
<td>246</td>
<td>13</td>
<td>1.06 (0.60 to 1.88)</td>
</tr>
</tbody>
</table>
Source population:
Individuals living in Denmark 1998-2016 (born 1975-1999)

Non-retinoid acne drugs
- New users: n=268,434
- Excluded users:
  Prior MS diagnosis (before cohort entry or same calendar year), n=213
  Age <10 years, n=1,554
  Not resident at cohort entry, n=9,474

Topical retinoids
- New users: n=134,881
- Excluded users:
  Prior MS diagnosis (before cohort entry or same calendar year), n=83
  Age <10 years, n=838
  Not resident at cohort entry, n=3,400

Systemic retinoids
- New users: n=77,082
- Excluded users:
  Prior MS diagnosis (before cohort entry or same calendar year), n=39
  Age <10 years, n=14
  Not resident at cohort entry, n=1,419

Three-way propensity score weighting
This article is protected by copyright. All rights reserved.
Unweighted

Weighted

Patients at risk, n
Systemic retinoids 75610 62258 47831 35945 26621 19884 14198 9523 4422 926
Topical retinoids 130560 97262 75238 57857 43467 32859 21232 11759 4775 734
Non-retinoids 252193 19281 152121 119873 92726 69211 51455 37126 22548 6884

Patients at risk, n
Systemic retinoids 73236 60423 46460 34913 25766 19123 13481 8866 4009 836
Topical retinoids 73032 50620 36955 27085 19859 15103 10384 6387 2911 445
Non-retinoids 72788 49319 34943 24919 18583 13244 9378 6055 3237 989

This article is protected by copyright. All rights reserved