Research Article

^{11}C-PK11195 plasma metabolization has the same rate in multiple sclerosis patients and healthy controls: a cross-sectional study

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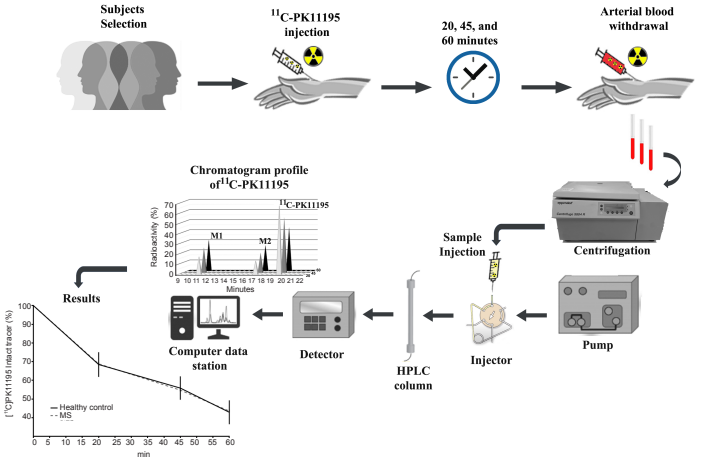
Graphical Abstract *Analysis of ^{11}C-PK11195 radiometabolites by high performance liquid chromatography shows the same metabolization rate in multiple sclerosis patients and healthy controls*



Abstract

^{11}C-PK11195 is a positron emitter tracer used for Positron Emission Tomography (PET) imaging of innate immune cell activation in studies of neuroinflammatory diseases. For the image quantitative analysis, it is necessary to quantify the intact fraction of this tracer in the arterial plasma during imaging acquisition (plasma intact fraction). Due to the complexity and costs involved in this analysis it is important to evaluate the real necessity of individual analysis in each ^{11}C-PK11195 PET imaging acquisition. The purpose of this study is to compare ^{11}C-PK11195 plasma metabolization rate between healthy controls and multiple sclerosis (MS) patients and evaluate the interference of sex, age, treatment, and disease phenotype in the tracer intact fraction measured in arterial plasma samples. ^{11}C-PK11195 metabolization rate in arterial plasma was quantified by high performance liquid chromatography in samples from MS patients (n = 50) and healthy controls (n = 23) at 20, 45, and 60 minutes after ^{11}C-PK11195 injection. Analyses were also stratified by sex, age, treatment type, and MS phenotype. The results showed no significant differences in the metabolization rate of healthy controls and MS patients, or in the stratified samples. In conclusion, ^{11}C-PK11195 metabolization has the same rate in patients with MS and healthy controls, which is not affected by sex, age, treatment, and disease phenotype. Thus, these findings could contribute to exempting the necessity for tracer metabolization determination in all ^{11}C-PK11195 PET imaging acquisition, by using a population metabolization rate average. The study procedures were approved by the Ethics Committee for Research Projects Analysis of the Hospital das Clinicas of the University of Sao Paulo Medical School (approval No. 624.065) on April 23, 2014.

Key Words: ^{11}C-PK11195; HPLC; kinetic modeling; metabolization; multiple sclerosis; neuroinflammation; PET imaging; PET tracer; radiometabolites

Chinese Library Classification No. R445; R364; R741

Introduction

^{11}C-PK11195 is an isoquinoline carboxamide labeled with carbon-11 radioisotope that specifically binds the 18 kDa translocator protein (TSPO) present in the mitochondria of cells (Shah et al., 1994; Trapani et al., 2013). This radiotracer promotes *in vivo* visualization and quantification of innate

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immune cell activation in studies of neuroinflammatory diseases by positron emission tomography (PET), for example multiple sclerosis (MS) (Best et al., 2019). ^{11}C-PK11195 is a first generation TSPO PET radiotracer and has the disadvantage of its low signal-to-noise ratio; however it is still used in many PET centers, having the advantage over the second TSPO tracer generation of not being influenced by the nucleotide polymorphism in the TSPO gene (Downer et al., 2020).

MS is an inflammatory and demyelinating disease of the central nervous system and can be classified as relapsing-remitting MS (RRMS), or progressive MS [primary progressive (PPMS) or secondary progressive (SPMS)]. MS was the first neuroinflammatory disease to be studied by TSPO PET imaging, as neuroinflammation assessment allows evaluation of disease progression and anti-inflammatory treatment efficacy (Downer et al., 2020).

MS is a widespread neuroinflammatory disease in the brain, making it challenging to find a "disease-free region" to use as a reference region for PET imaging full quantification (kinetic-modeling); therefore, an arterial plasma "input-function" and radioactive metabolite determination are needed (Schuiternaker et al., 2007). Radiometabolite analysis is complex, costly, and time consuming and working with carbon-11 labeled tracers introduces an additional challenge due to their short half-life.

This study aims to compare the ^{11}C-PK11195 metabolization rate in healthy controls (HC) and multiple sclerosis patients and also in the sample stratified by sex, age, treatment, and disease phenotype.

Participants and Methods

Participants

The present cross-sectional study was performed in agreement with the principles outlined in the *Declaration of Helsinki* and reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement (**Additional file 1**). The Ethics Committee for Research Projects Analysis of the Hospital das Clinicas of the University of Sao Paulo Medical School approved the procedures (approval No. 624.065) on April 23, 2014 (**Additional file 2**) and informed consent was obtained from all individual participants included in the study (**Additional file 3**). Based on earlier ^{11}C-PK11195 studies in animal model (de Paula Faria et al., 2014) and clinical data (Sucksdorff et al., 2017), the power test (80% power and 95% confidence), using analysis of variance, estimated the minimum of five people in each group to reveal difference between RRMS and progressive phenotypes in the imaging analysis. Considering the large variability of MS compared to animal models, the rarity of progressive phenotypes, and exploratory nature of the study, we defined a sample size of 15 in the RRMS, PMS and HC groups in a way to allow stratified analysis in these groups.

Fifty-one patients with MS were enrolled between October 2017 and December 2018. Inclusion criteria were diagnosis of MS according to current McDonald criteria (Thompson et al., 2018), age between 18 and 65 years and availability of detailed medical history. At inclusion, a trained neurologist evaluated disability using the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). Exclusion criteria were corticosteroids or benzodiazepines use within 4 weeks prior to the protocol (Schocke et al., 2003; Turkheimer et al., 2015), contraindication to radial arterial line placement (Berg et al., 2014) and presence of comorbidity considered a limitation to undergo PET imaging acquisition. Twenty-four age- and sex-matched HCs were recruited from the community. One HC and 1 patient were excluded from this analysis because technical problems during plasma sample analysis.

Plasma samples of 73 subjects (23 HCs and 50 MS patients) were used in this study: 46 women (16 HCs) and 27 men (7 HCs) aged 19–62 years (**Table 1**). Arterial blood samples were collected during a PET imaging research protocol using ^{11}C-PK11195 from healthy subjects and patients recruited in the MS outpatient clinic of the University Hospital.

Table 1 | **Demographic data of included participants**

	HC (*n* = 23)	RRMS (*n* = 30)	PPMS (*n* = 12)	SPMS (*n* = 8)	*P*-value
Age[&] (yr)	42.2±12.5 (22–59)	35.7±7.6 (19–49)	53.2±6.3 (40–62)	43.4±8.4 (48–55)	0.0001[*]
Female/male (*n*)	7/16	9/21	5/7	4/4	0.258[$]
EDSS[&]	–	2.7±1.4 (1–6)	6.2±0.8 (4–7.5)	6.5±0.5	0.0001[#]

[&]Data are expressed as the mean ± SD (range). *One-way analysis of variance with Bonferroni correction; $Fischer's exact test; # Kruskal-Wallis. EDSS: Expanded Disability Status Scale; HC: healthy control; MS: multiple sclerosis; PPMS: primary progressive MS; RRMS: relapsing-remitting MS; SPMS: secondary progressive MS.

^{11}C-PK11195 metabolization analysis

^{11}C-PK11195 was produced by the institutional cyclotron and radiopharmacy and the final product was approved by quality control for human use.

A trained physician punctured the radial artery and arterial blood (4 mL/sample) was collected at 20, 45, and 60 minutes post ^{11}C-PK11195 injection (385.54 ± 0.47 MBq).

Blood samples were centrifuged for 3 minutes at 3500 × *g*, then the plasma was separated and mixed with acetonitrile (2 mL) for protein precipitation. The mixture was centrifuged for 1 minute at 3500 × *g* and the supernatant was filtered on a 0.45 µm Millex PVDF filter (Merck Millipore, Darmstadt, Germany). Plasma filtrate was injected into a high performance liquid chromatography (HPLC) (Agilent 1260 Infinity II LC System, Agilent Technologies, Waldbronn, Germany), with a C18 Luna® column (Phenomenex, 250 × 4.6 mm, 5 µm) using ethanol: water (60:40) as mobile phase, at a flow rate of 2.5 mL/min. Fractions were collected each one minute for 22 minutes and measured in a gamma counter (Hidex, Turku, Finland).

Statistical analysis

Results are presented as mean, standard deviation (SD), and sample size (*n*) for each time point: 20 minutes, 45 minutes, and 60 minutes after radiotracer injection (SPSS Statistics 20 Software, IBM Corp., Armonk, NY, USA and GraphPad Prism 6 Software, GraphPad Software Inc., La Jolla, CA, USA). The data passed the Kolmogorov-Smirnov test for normality and therefore parametric tests were applied in the analysis, except for the EDSS scale, for which the Kruskal-Wallis test was performed. The HPLC data were analyzed by a general linear model for one dependent variable (% of ^{11}C-PK11195 intact fraction), and fixed factors with different combinations, being: sex, groups, and time points; HCs, MS, and time points; categorized age and time points; treatment and time points. Groups were HCs, RRMS, PPMS, and SPMS. Age was categorized into 3 groups: between 19–35, 36–50, and 51–62 years. Treatment was categorized into 5 groups: no treatment (naïve or without treatment for more than 4 months), first-line injectable therapies (beta interferons and glatiramer acetate), oral therapies (fingolimod and dimethyl fumarate), natalizumab, and classic immunosuppressants (azathioprine and methotrexate) (Hauser and Cree, 2020). Statistical analysis for the age difference between groups was conducted by one-way analysis of variance. Bonferroni correction for multiple comparisons was performed when appropriate. Differences between sexes were analyzed by the Fisher´s exact test. *P* values < 0.05 were considered statistically significant.

Results

Demographic data and ^{11}C-PK11195 metabolization profile

Demographic data are presented in **Table 1**. The HPLC chromatogram profile of ^{11}C-PK11195 and its metabolites measured at 20, 45, and 60 minutes post tracer injection is presented in **Figure 1**, showing the decrease in ^{11}C-PK11195 intact fraction and increase in metabolites over time. ^{11}C-PK11195 intact fractions in each group and at 20, 45, and 60 minutes post ^{11}C-PK11195 injection are presented in **Table 2**.

Table 2 | ^{11}C-PK11195 intact fraction in each group, including stratified groups at 20, 45, and 60 minutes post ^{11}C-PK11195 injection

	20 min	45 min	60 min
Tracer intact fraction by sex			
Male	68.97±6.41 (27)	54.49±6.42 (27)	43.91±5.76 (26)
Female	68.53±5.86 (46)	55.43±5.50 (44)	42.95±5.83 (42)
Tracer intact fraction in healthy controls and MS patients			
Healthy control	68.44±6.62 (23)	55.84±6.26 (22)	42.87±6.62 (21)
MS patients	68.81±5.80 (50)	54.73±5.77 (49)	43.52±5.53 (47)
Tracer intact fraction in the different MS phenotypes			
RRMS	69.77±5.71 (30)	55.31±5.70 (29)	43.90±5.41 (28)
PPMS	66.45±5.22 (12)	53.57±6.11 (12)	41.44±5.01 (11)
SPMS	68.76±6.64 (8)	54.37±5.28 (8)	45.02±6.51 (8)
Tracer intact fraction by age (yr)			
19–34	69.83±5.70 (23)	55.36±5.96 (23)	43.93±5.89 (22)
35–50	68.35±6.22 (31)	54.97±5.54 (30)	42.90±5.32 (29)
51–62	67.88±6.21 (19)	54.88±6.47 (18)	43.32±5.78 (17)
Tracer intact fraction by treatment type			
No treatment	67.84±6.45 (11)	55.55±8.85 (11)	46.15±6.09 (11)
First-line injectable therapies	70.24±6.66 (15)	54.55±6.3 (15)	42.73±4.58 (14)
Oral therapies	69.16±4.80 (9)	56.69±4.63 (8)	45.09±6.84 (8)
Natalizumab	69.21±4.70 (11)	54.64±4.58 (11)	42.09±4.58 (11)
Classic immunosuppressants	64.24±5.13 (4)	49.46±6.75 (4)	38.56±3.86 (3)

Data are expressed as the mean ± SD (*n*). MS: Multiple sclerosis; PPMS: primary progressive multiple sclerosis; RRMS: relapsing-remitting multiple sclerosis; SPMS: secondary progressive multiple sclerosis.

^{11}C-PK11195 metabolization by sex and age

Comparisons of radiotracer metabolization between women and men are shown in **Figure 2A**. No statistical differences were found at any time point ($F_{(1,200)}$ = 0.600, P = 0.440). Considering the effect of age, the three stratified groups (19–34, 35–50, and 51–62 years) presented the same tracer metabolization rate ($F_{(2,203)}$ = 0.646, P = 0.525; **Figure 2B**).

^{11}C-PK11195 metabolization in multiple sclerosis patients and HCs

The rates of ^{11}C-PK11195 metabolization in healthy volunteers and MS patients are given in **Figure 2C**. At all time points, the comparisons of radiotracer rates between volunteers with or without MS showed no statistical differences ($F_{(1,200)}$ = 0.292, P = 0.589). Stratifying the MS group by disease phenotypes (**Figure 2D**), there were also no significant differences at any time points ($F_{(3,188)}$ = 1.306, P = 0.274). Considering the effect of treatment, no statistical differences were found at any time point ($F_{(10,194)}$ = 0.481, P = 0.901, **Figure 2E)**.

Discussion

This study compared ^{11}C-PK11195 metabolization rates in HCs and MS patients, showing no differences between them at any of the studied time points after tracer injection. There were no significant differences between sexes, age categories, MS phenotypes and treatment categories.

The metabolization rate for ^{11}C-PK11195 has previously been studied previously. Greuter et al. (2005) analyzed the plasma of 10 healthy volunteers and showed ^{11}C-PK11195 intact fractions of 57.5 ± 6.4% at 20 minutes post tracer injection and 45.2 ± 9.4% at 59 minutes. Our data show a slower

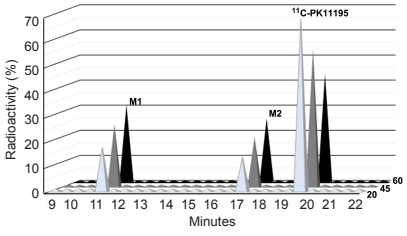

Figure 1 | HPLC chromatogram profile of ^{11}C-PK11195 intact fraction and its radiometabolites (M1 and M2) at 20, 45, and 60 minutes post tracer injection.

Note that ^{11}C-PK11195 intact fraction decreases with time (20 minutes in light grey, 45 minutes in dark grey, and 60 minutes in black) while the radiometabolites fractions increase with time.

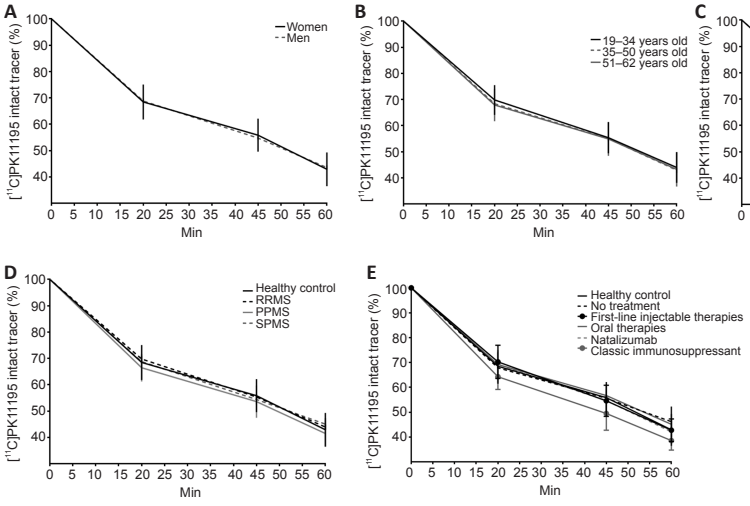

Figure 2 | ^{11}C-PK11195 metabolization rate over time in the different groups analyzed.

(A) Tracer metabolization by sex. (B) Tracer metabolization by age. (C) Tracer metabolization comparing healthy controls and multiple sclerosis (MS) patients. (D) Tracer metabolization in the different MS phenotypes and healthy controls. (E) Tracer metabolization by treatment categories. PPMS: Primary-progressive MS; RRMS: relapsing-remitting MS; SPMS: secondary-progressive MS.

metabolization rate at 20 min, but similar rate at 60 minutes. In Alzheimer's disease, samples of 10 patients were analyzed (Roivainen et al., 2009), and the ^{11}C-PK11195 fraction at 20 minutes post tracer injection was 76.2 ± 6.1% and at 50 minutes was 57.8 ± 10.9%. The authors suggested that the slower metabolization rate compared to Greuter et al. (2005) is due to sample age (61–82 years old *vs*. 18–77 years old). The explanation about age interference is coherent, however our data did not show an age effect, which could be due to our older group (51–62 years) being younger than the study sample of Roivainen et al. (2009), which also mentioned unpublished data of patients with coronary artery disease, with slightly different results from AD patients. Another study (Pike et al., 1993) evaluated ^{11}C-PK11195 metabolization rate in cardiac embolic infarct patients and MS patients with a slower metabolization rate at 60 minutes (55 ± 7%) compared with our study. In that study the sample was small (n = 15) and no separation was made between cardiac embolic infarct patients and MS patients, preventing a direct comparison of MS patient data. De Vos et al. (1999) evaluated 5 subjects (2 HCs and 3 stroke patients) aged from 50–75 years and found 78.0 ± 3.3% of intact ^{11}C-PK11195 in arterial plasma after 20 minutes post tracer injection, but there was no comparison between the rates of HCs and stroke patients. Jučaite et al. (2012) described an intact tracer fraction of 70 ± 1% at 60 minutes post tracer injection in plasma of 6 healthy young men (21–31 years old), this being a slower metabolization rate compared to our results, but with high variability in their small sample (range from 60% to 77%). Although the metabolization rate was not the same as in our study, the HPLC profile from parent tracer and metabolites separation were quite similar.

There was no differences in ^{11}C-PK11195 metabolization rate between schizophrenia patients (n = 10) and HCs (n = 10) (van Berckel et al., 2008), which is in agreement with our study comparing MS patients and HCs. In the study of Kropholler et al. (2009), the ^{11}C-PK11195 metabolization rate of rheumatoid arthritis patients (n = 9), was similar to other studies and, therefore, the authors concluded that the correction of the plasma input curve for metabolites did not change the results, and that based on practical considerations, the quantification of ^{11}C-PK11195 uptake could be made without plasma metabolite correction in this population.

All previous studies used a limited number of subjects, which does not allow for sensitivity analysis (such as sex and age), which was possible with our larger sample size, generating more robust comparisons between different groups and, thus, we demonstrated that ^{11}C-PK11195 metabolization rate is similar in HCs and MS patients, with no statistical differences between them at any of the studied time points after tracer injection. Furthermore, there were no significant differences between sexes, age, and treatment categories, and MS phenotypes.

Higher motor disability, shown by EDSS, also did not influence tracer metabolization, since progressive MS phenotypes (PPMS and SPMS) have a higher EDSS and metabolization did not differ from the HC and RRMS groups.

Treatment with immunosuppressive drugs may alter plasma metabolization rate of drugs (Elbarbry et al., 2008; Vanhove et al., 2017), however, no studies were found on the effect on PET tracer metabolization rate. In our treatment categories, no differences were found in ^{11}C-PK11195 metabolization when compared with either the control group or with the group without treatment. Since the variability in treatment type was high in our sample, even when classifying them by categories, the sample size of each group was relatively small, especially in the classic immunosuppressant groups (n = 4), which could limit a robust conclusion about treatment effects.

It is important to observe that tracer metabolism apparently does not change in the presence of multiple sclerosis, which allows the use of metabolization data from healthy subjects for ^{11}C-PK11195 PET image analysis, i.e., the possibility of using a metabolization average rate from population data without the necessity of performing metabolite analysis in each ^{11}C-PK11195 PET imaging acquisition. The metabolization rate should, however, be validated in other countries, to observe whether ^{11}C-PK11195 metabolization is influenced by population characteristics, although comparisons with previous studies already indicate similar rates. More studies considering treatment effect would also be desirable.

The limitation of this study is to be a single-center study, with low variability in race and population geography. The sample size, although higher than several discussed studies, is small when variables were categorized, especially treatment categories. Some data are missing within the different time points due to the technical complications (as artery blockage during blood within drawn).

In conclusion, ^{11}C-PK11195 metabolization has the same rate in patients with multiple sclerosis and HCs, which was not affected by sex, age, treatment, or disease phenotype. These data could contribute to exempting the necessity for tracer metabolization determination in all ^{11}C-PK11195 PET imaging acquisition by using a population metabolization average, thus, reducing the cost and complexity of metabolite correction in the arterial input function for kinetic modeling analysis.

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Conflicts of interest: *The authors declare that they have no conflict of interest related to this paper. No conflicts of interest exist between GE Healthcare and publication of this paper.*

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Institutional review board statement: *The study was approved by the the Ethics Committee for Research Projects Analysis of the Hospital das Clinicas of the University of Sao Paulo Medical School (approval No. 624.065) on April 23, 2014.*

Declaration of participant consent: *The authors certify that they have obtained all appropriate participant consent forms. In the forms the participants have given their consent for their images and other clinical information to be reported in the journal. The participants understand that their names and initials will not be published and due efforts will be made to conceal their identity.*

Reporting statement: *This study followed the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.*

Biostatistics statement: *The statistical methods of this study were reviewed by the biostatistician of Hospital das Clinicas of the University of Sao Paulo Medical School, in Brazil.*

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Data sharing statement: *Datasets analyzed in this study are available from the corresponding author on reasonable request.*

Plagiarism check: *Checked twice by iThenticate.*

Peer review: *Externally peer reviewed.*

Additional files:

Additional file 1: *STROBE checklist.*
Additional file 2: *Ethical Approval Documentation (Portuguese).*
Additional file 3: *Model consent form (Portuguese).*

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C-Editors: Zhao M, Zhao LJ, Li CH; T-Editor: Jia Y

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	4 and 5
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5 and 6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	6
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	6
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Tables 1 and 2
		(b) Give reasons for non-participation at each stage	5
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 2
Outcome data	15*	Report numbers of outcome events or summary measures	Table 2

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
		(*b*) Report category boundaries when continuous variables were categorized	Table 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Table 2
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-11
Generalisability	21	Discuss the generalisability (external validity) of the study results	11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.