Parkinsonism and Related Disorders 64 (2019) 90–96

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

# Anemia in men and increased Parkinson's disease risk: A population-based large scale cohort study

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ARTICLE INFO

*Keywords:*
Parkinson's disease
Anemia over time
Cohort study

ABSTRACT

*Objective:* To evaluate the association between anemia and Parkinson's disease risk (PD) in men and women.
*Methods:* A population-based cohort of 474,129 individuals (aged 40–79 years at date of first Hb test, 47.4% men) with repeated Hb levels was derived from a large Healthcare Maintenance Organization that serves 2 million citizens in Israel (study-period 1.1.1999–31.12.2012). An annual anemia indicator [Hb levels (g/dL) for men < 13; for women < 12.0] was assessed for each individual and they were followed from first Hb test until the date of PD incidence, death or end of the study. Cox-proportional hazards models, stratified by sex and age, with time-dependent anemia covariate were used to estimate adjusted Hazard Ratio with 95% of confidence intervals (HR, 95%CI) for PD.
*Results:* During a mean follow up of 8.8 ± 3.9 years (7.0 ± 3.6 for men and 7.9 ± 4.1 for women), 2427 incident PD cases were detected. Cumulative PD incidence at ages over 65 years was 3.3%. The mean levels of Hb at baseline was 14.8 ± 1.1 g/dL among men; 12.8 ± 1.1 g/dL among women. Anemia was associated with significant PD risk among men, age-pooled HR = 1.19 (95%CI: 1.04–1.37), with the highest risk between ages 60–64 years [HR = 1.41 (95%CI: 1.03–1.93)]. Anemia was not associated with PD risk among women across all age-groups. The age-pooled HR for women was 1.02 (95%CI 0.95–1.09).
*Conclusions:* The finding that anemia was associated with PD risk in men, especially in middle age, warrants further investigations on common pathophysiologic processes between Hb abnormalities and brain dysfunction.

## 1. Introduction

A number of epidemiological studies have recently highlighted the association between hemoglobin (Hb) levels and Parkinson's disease (PD) risk [1–4]. One study found a significantly increased risk for PD from 10.3 to 34.9/10,000 person-years as Hb concentration in peripheral blood increased from 14 to ≥16 g/dL (relative Hazard Ratio [HR] 3.2; 95% confidence interval [CI] 1.2–8.9) [3]. Other studies, however, reported that anemia (Hb < 13.0 g/dL for men and Hb < 12.0 g/dL for women) was associated with increased PD risk suggesting that anemia may be an early biomarker of PD. The study by Savica et al. found that anemia that had started 20–29 years before the PD onset was a significant risk marker for PD (OR = 4.26; 95%CI: 1.55–11.69) [1]. The study by Hong et al. found an increased PD risk in new anemic patients (HR = 1.36; 95%CI: 1.22–1.52)[4]; and Logroscino et al. reported an increased risk of PD only among men for whom there were data on a higher number of blood samples (> 5) close to the onset of PD (HR = 2.84; 95%CI: 1.00–8.07)[2].

The current study aimed at examining the association between anemia and PD risk. We used repeated individual-level Hb measurements during 14 years in a population-based large-scale cohort, all members of a large Israeli Healthcare Organization- Maccabi Healthcare Services (MHS) that covers 2 million individuals.

## 2. Methods

### 2.1. Study population

The participants of this population-based cohort study were members of Maccabi Healthcare Services (MHS) with Hb test results. MHS is the second-largest Healthcare organization in Israel and covers 25% of

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https://doi.org/10.1016/j.parkreldis.2019.03.010
Received 5 August 2018; Received in revised form 12 March 2019; Accepted 16 March 2019

the population. They were 40–79 years of age and free of PD at first Hb test between January 1999 and December 2012 (study period). The cohort included 474,129 individuals who had more than two annual records of Hb levels during the study period. They were prospectively followed from the first date of their Hb test (start of follow up) until the date of PD diagnosis, death, leaving MHS or study closure (end of follow up), whichever occurred first.

### 2.2. Assessment of anemia over time

Hb levels were measured by the common cell blood count test that is routinely measured in current clinical practice in Israel. Data on Hb measurements were collected from computerized laboratory databases of MHS during the study period (numbering a total of 4,179,941 Hb tests for the entire cohort, on average 6.0 ± 3.0 for men and 6.0 ± 3.1 for women who developed PD and 5.9 ± 3.2 for men and 6.0 ± 3.1 and 6.4 ± 3.5 who did. We calculated the mean annual Hb levels for each individual where the first mean annual Hb value was defined as the *baseline level.* More than 60% of cohort individuals (63% men and 68% women) had full information on Hb levels for their entire follow up period (e.g. 2,3,4, 5 repeated annual Hb levels for 2,3,4,5 years of follow up, respectively). For the rest of the individuals, we followed a single imputation approach in order to avoid potential biases which might arise from missing values (i.e., selection and information) [5,6]. We applied one of the following methods depending on the missing Hb values pattern: regression, last observation carried forward or first observation carried backward. Continuous Hb levels for each year for each individual were categorized into anemia (yes/no) by applying World Health Organization cutoffs for clinical anemia, which are < 13.0 g/dL for men and < 12.0 g/dL for women [7].

### 2.3. PD assessment

PD was assessed by using an established anti-parkinsonian drug (APD) tracer algorithm which was previously applied and described in detail elsewhere [8]. Briefly, the algorithm detected PD cases based on purchases of any APD among 17 generic APDs (categorized as dopaminergic agents, ATC code N04B) and available in Israel during the study period. Based on different combinations of the following four criteria, the algorithm (see appendix 1) determines a level of probability of PD incidence *(definitive, probable, or possible):* (a) APD purchase profiles, (b) age at first APD purchase, (c) APD purchase intensity—number and continuity of purchases, and (d) length of follow up period (calculated from the date of first purchase APD until date of death, date of leaving the MHS or end of study -December 31, 2012).

In this study, the algorithm was refined by several selection criteria (e.g., excluding patients with fewer than three consecutive monthly purchases of APD and treatment by bromocriptine, cabergoline or amantadine only) and validated twice [9].

First validation was performed through the comparison of the algorithm assessment PD of 141 cases which were also treated at a private clinic of a neurologist specializing in movement disorders (treated as the gold standard diagnosis) during 1999–2012. For those patients, the algorithm positive predictive value (PPV) was 93% (131/141). Second validation was performed through the comparison of the algorithm assessment PD of 2247 cases (entire cohort) to PD diagnosis records of MHS in medical files and outpatients' visits (treated as the gold standard for this validation) using International Classification of Diseases – 9th Revision, (ICD-9-CM code 332.0). The codes were recorded by health care providers (e.g., neurologists, family physicians, nurses and etc.). In this validation, the algorithm PPV for the entire cohort was 89%, (1997/2247) however for definite PD was 100% (1700/1700). In order to assure the exclusion of restless leg syndrome (RLS) from our PD cohort (since this illness is also treated by APD), we reviewed all clinical records of the 2427 incident cases for RLS diagnosis, given during a visit to either a physician, a nurse, an outpatient clinic, etc. The records related to RLS diagnoses are very accurate since an approval diagnosis by a neurologist is required for purchasing APD by RLS patients. We found that all PD cases in our cohort were free of RLS diagnosis. The main analysis of PD incidence was carried out for all levels of PD certainty (definite, probable or possible).

### 2.4. Statistical analysis

*Main Analyses:* Time-dependent Cox models were used to estimate the effect of anemia over time on the risk of PD, in 5-year age at first Hb test groups by sex. The time scale was years of follow-up lagged one year prior to the last year of follow-up. The time-dependent variable was annual anemia (yes/no). All models were adjusted for age at first Hb test as a continuous variable. We first estimated HRs for each sex and age group, and then the log HRs for the different age groups in each sex were pooled by a random effects model (weighted by the standard error) to estimate an age-pooled HR.

*Sensitivity Analyses:* We fitted further Cox modeling as follows: First, we applied a stratified model by follow-up time (≤5 years and > 5 years) to account for a potential duration effect. Second, the 1-year lag time was replaced by 5 years to allow for a biologically meaningful latency time window where PD occurred before the onset of motor disturbances [1]. Third, we examined the risk of PD associated with Hb level as a three categories variable: anemia, normal range and high level (< 13.0 g/dL, 13.0–17.0 g/dL, and ≥17.0 g/dL for men and < 12.0 g/dL, 12.0–16.0 g/dL, and ≥16.0 g/dL for women). Finally, the modeling was restricted to *definite* PDs only.

Statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, IL) SAS version 9.4 (SAS Institute, Cary, NC) and MetaXL version 5.3.

### 2.5. Ethics

This study was approved by the Helsinki Committee (Institutional Review Board [IRB]) of the Tel Aviv Sourasky Medical Center (No. 0281-13-TLV) and the Helsinki IRB of MHS-Assuta Medical Center (No. 2013052). The patients' personal ID numbers that were used to link between different databases were encrypted prior to delivery to the investigators in order to ensure participant anonymity. The study involved no direct interaction with patients, therefore, informed consent was not required by the IRB.

## 3. Results

### 3.1. General characteristics

The cohort included 474,129 individuals, (47.4% men) with a mean follow-up of 8.8 ± 3.6 years (Table 1). The cumulative follow-up was 4,134,957 person-years consisting of 1,967,082 person-years for the men and 2,167,845 person-years for the women. The average age at first Hb test was 48.7 ± 9.3 years for men and 47.7 ± 9.7 years for women, while 30% of the men and 25% of the women were over the age of 50 years. The distribution of annual level of Hb at baseline was higher among men compared to women (14.8 ± 1.1 g/dL and 12.8 ± 1.1 g/dL, respectively). Individuals who developed PD had a slightly shorter follow up period, a similar number of annual Hb tests and similar Hb levels at baseline, as compared to individuals who did not (Supplementary Table 1).

### 3.2. Rates of PD incidence

During the study period, 2427 (0.5%) incident PD cases were detected, reflecting an incidence density rate of 7.1 per 10,000 person-years for men and 4.7 per 10,000 person-years for women. Table 2 presents age-specific PD cumulative incidence rates which increased with age for both sexes: they ranged from 0.07% to 4.4% between the youngest and oldest age groups, respectively, for men (3.3% for age ≥65 years), and from 0.05% to 3.1% at equivalent age groups (2.6%

**Table 1**
Characteristics of the study cohort ($n$ = 474,129); by sex.

| Characteristic | Men (n = 224,948) | Woman (n = 249,181) |
|---|---|---|
| **Age (years) at first Hb measurement** | | |
| Mean ( ± SD) | 48.7 (9.3) | 47.7 (9.7) |
| Median (IQR) | 45.0 (41.1–53.6) | 42.6 (40.6–52.2) |
| **Follow up period (years)** | | |
| Mean ( ± SD) | 8.8 (3.8) | 8.7 (3.9) |
| Median (IQR) | 9.0 (6.0–12.0) | 9.0 (5.0–12.0) |
| Person-years | 1,967,082 | 2,167,845 |
| **Hb levels (g/dL) at baseline** | | |
| Mean ( ± SD) | 14.8 (1.1) | 12.8 (1.1) |
| Median (IQR) | 14.9 (14.2–15.5) | 12.9 (12.2–13.6) |
| Range (min.-max.) | 9.9–19.7 | 8.5–17.6 |
| **PD cases, n (%)** | 1416 (0.6) | 1011 (0.4) |
| **Death cases, n (%)** | 12,945 (5.8) | 9894 (4.0) |
| **Left MHS, n (%)** | 7746 (3.4) | 9277 (3.7) |

Abbreviations: Hb, hemoglobin; SD, standard deviation; IQR, interquartile range; MHS, Maccabi Health Services.

for age ≥65 years) for women. The average age at first APD treatment was similar for both men and women (70.1 ± 9.6 years and 70.6 ± 9.9 years, respectively). The PD incidence was 1.2% for the men with anemia at baseline compared to 0.6% for those without. However, the PD incidence exhibited a similar rate among the women with and without anemia at baseline (0.3% vs 0.4%, respectively).

### 3.3. Anemia and PD risk

*Main Analyses:* There was a trend of increased HR of PD in all age groups associated with anemia, for men (Fig. 1A). All estimates of HR of PD associated with anemia were over unity (range: 1.02–1.91, but mostly non-significant). A significantly increased risk of 41% was found for men aged 60–64 years (HR = 1.41; 95%CI 1.03–1.93). The age-pooled HR for men was 1.19 (95%CI 1.04–1.37), and it demonstrated a significantly increased PD risk of almost 20% for those with anemia.

The association between anemia and PD risk among women was non-significant in all age groups (Fig. 1 B). The range of HR was 0.80–1.33, with the highest HR for the age 65–69 years (HR = 1.33; 95%CI 0.96–1.85). The age-pooled HR for women was 1.02 (95%CI 0.95–1.09), indicating a non-significant PD risk associated with anemia.

*Sensitivity analyses:* All sensitivity analyses yielded results in a similar direction, where the associations between anemia and PD risk were significant only in men. For example, in the 5-year lagged analysis, the age-pooled HR (95%CI) was 1.20 (1.02–1.41) for men and 0.97 (0.83–1.14) for women. Additionally, when Hb levels were considered as three categories variable: anemia, normal range and high level: For men with anemia vs. normal range or high level, the age-pooled HRs (95%CIs) were 1.11 (0.98–1.25; $P$ *value* = 0.06) and 1.19 (0.98–1.44; $P$ *value* = 0.07) respectively. For women with anemia vs. normal range or high level, the age-pooled HRs (95%CIs) were non-significant 0.98 (0.84–1.15) and 0.92 (0.77–1.10), respectively.

**Table 2**
Age-specific PD cumulative incidence rates[a] among the study cohort; by sex.

| Age group at first blood test (years) | [N total (% PD)] | |
|---|---|---|
| | Men (n = 224,948) | Women (n = 249,181) |
| 40–44 | 111,983 (0.07) | 145,488 (0.05) |
| 45–49 | 35,965 (0.2) | 30,134 (0.1) |
| 50–54 | 27,420 (0.5) | 23,685 (0.4) |
| 55–59 | 17,125 (0.9) | 15,145 (0.6) |
| 60–64 | 13,917 (2.0) | 14,061 (1.2) |
| 65–69 | 9367 (3.0) | 8999 (2.3) |
| 70–74 | 5899 (4.7) | 6807 (2.8) |
| 75–79 | 3272 (4.4) | 4862 (3.1) |

[a] The number of new PD cases divided by the total number of individuals for specific age-groups at first blood test; during the study period.

## 4. Discussion

The results of this population-based large-scale cohort study that included 4,179,941 repeated individual–level Hb measurements from 474,129 individuals showed that anemia was associated with increased PD risk among men but not women. The categorization of Hb levels as having/not having anemia when studying PD risk demonstrated that the development of anemia over time was associated with a significantly increased PD risk of 19% among men over age 40 years, with the highest risk of 40% among middle-aged men (age 60–64 years). Our findings strengthen those of Savica et al. who showed that PD begins decades before clinical manifestations are apparent [10]. The disease may involve Hb metabolism, and the association between Hb and PD might be of common pathophysiologic processes between Hb abnormalities and brain dysfunction.

We propose that bone marrow function may become impaired throughout the course of the disease in its prodromal stage. Recent studies recognized PD as a systemic disease wherein a systemic inflammatory response has been implicated in its pathogenesis [11], in addition to chronic neuroinflammation of the nervous system [12]. In support of this hypothesis, evidence showing inflammatory cytokines were found to be significantly increased in the peripheral blood of patients with PD [13], which can lead to decreased erythropoietin, and finally cause anemia. Damage by cytokines was shown to gradually increase with disease duration [14], leading to the decline of Hb levels over time.

There are several alternative explanations for the association between anemia and PD risk other than systemic inflammation. Possible mechanisms may include oxidative stress and systemic iron metabolism. It is generally accepted that alterations in oxidative stress contribute to the development of PD [15]. Recent evidence showed that peripheral mitochondrial function in platelets and white blood cells (lymphocytes) is consistently impaired in PD patients compared to controls [16–18]. and that the erythrocytes of PD patients tend to undergo eryptosis [19] due to excessive oxidative stress [20], resulting in the decline of Hb levels. Systemic iron metabolism has also been investigated, and several studies found a protective effect of high serum iron levels on PD [21–23] and even on disease severity in patients with PD [24].

Whether or not diet has some role in Hb levels also needs to be considered. New PD patients showed alterations in the energetic profile, such as weight loss, reduced caloric intake, and increased energy requirement, which may subsequently play a possible influence on the Hb level [25,26] or might reflect malnutrition and pathophysiological changes in the gastrointestinal tract in the prodromal pathogenesis of PD.

Our age-pooled analyses showed a significantly increased PD risk for men, but not for women, demonstrating a host of potential contributing modulatory effects of specific sex-related differences in the pathogenesis of PD [15]. Additionally, anemia is more common in women than in men, and may be related to gynecological conditions such as menstrual cycle irregularities or uterine fibromas [1,27]. Since in this study the information on such comorbidities was not available, some caution is required in extrapolating these findings. However, these findings are novel, since no earlier follow-up study had investigated this association separately for men and women, and a search for its basis may yield new clues to the pathogenesis of PD.

The strengths of this study include: 1) the use of substantial data on individual-level repeated measurements of Hb levels in a community-based cohort 2) the use of Cox modeling with anemia as a time-dependent covariate which enabled examination of the real-life temporal effect of Hb levels on preclinical phases of PD; 3) the use of stratified analyses by sex and refined 5-year age categories which enabled examination of the modifying effect of sex and age groups on PD risk associated with anemia in an empowered analysis.

Study limitations concern drug tracer assessment of a PD case which could identify non- PD patients due to the difficulty in differentiating

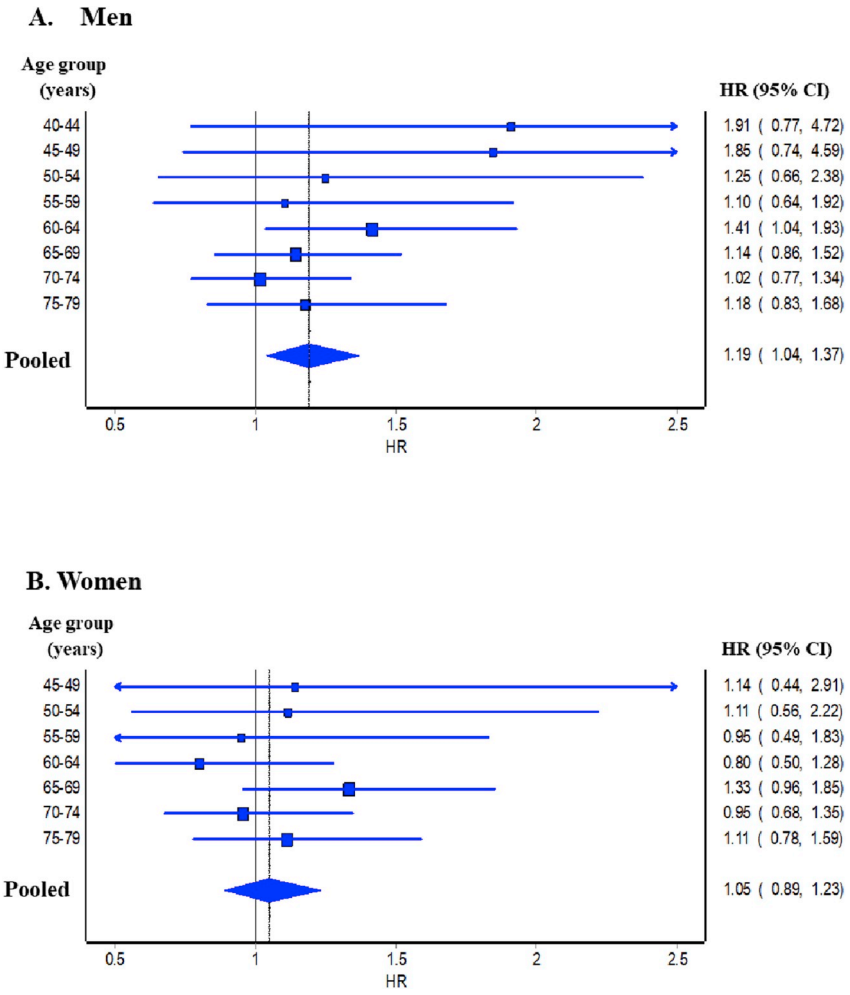


**Figure 1.** Pd risk associated with annual measurements of anemia; by sex and age at first Hb test.

Parkinson plus, secondary parkinsonism and some age-related syndromes from PD, solely according to the treatment. However, these cases should be rare and the impact of these limitations is reduced, due to the refined criteria and double validation. Second, information on confounders such as ethnicity, smoking status, coffee intake, body mass index, genetic factors, comorbidities which might confound the Hb-PD association, was not available for analysis in this study. However, the results of a positive association between anemia and PD risk in previous studies which adjusted for potential confounders in their analyses, such as smoking risk [2], coffee intake risk [2] or comorbidities [4] were similar to ours. It would be worthwhile to further evaluate the effects of Hb on PD risk-adjusted for those potential confounders. In addition, although the MHS insure 2 million of the Israeli population, and according to the National Security reports they are similar to the general Israeli population in terms of age, sex, family status, and place of residence, our results may not be representative of the general Israeli population.

## 5. Conclusion

Our findings of increases in PD risk associated with anemia among men support the hypothesis that Hb levels may serve as a marker of the risk of developing PD. Given that Hb is frequently measured in current clinical practice, these results may have important translational consequences for identifying individuals at a prodromal phase of PD. Other longitudinal cohort studies are required to further elucidate the acceptable range of Hb levels changes throughout life, to define a time frame in which future use of biomarkers and risk stratification may detect PD, and how these changes affect PD.

## Authors' contribution

Dr. Violetta Rozani was responsible for designing the study, data analysis, data interpretation, and for drafting the manuscript.

Prof. N. Giladi was responsible for the conceptualization of the study, data interpretation and revising the manuscript.

Prof. T. Gurevich-responsible for designing and revising the manuscript.

Dr. B.El-Ad- was responsible for the conceptualization of the study and revising the manuscript.

Mrs. J. Tzamir-was responsible for data collection and revising the manuscript.

Mrs. Beatriz Hemo-was responsible for data collection and revising the manuscript.

Prof. C. Peretz-was responsible for designing and conceptualization of the study, data analysis, and interpretation, and drafting the manuscript.

## Acknowledgment

Esther Eshkol is thanked for editorial assistance.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2019.03.010.

## Author disclosures

Dr. V. Rozani – reports no disclosures. Prof. N. Giladi-serves as a member of the Editorial Board for the Journal of Parkinson's Disease. He serves as a consultant to Teva-Lundbeck, IntecPharma, NeuroDerm, Armon Neuromedical Ltd\Dexel, Monfort and Lysosomal Therapeutics Inc. He Received payment for lectures at Teva-Lundbeck, Novartis, UCB, Abviee, Shaier, and Genzyme. Prof. Giladi received research support from the Michael J. Fox Foundation, the National Parkinson Foundation, the European Union 7th Framework Program and the Israel Science Foundation as well as from Teva NNE program, LTI, and Abviee and CHDI.

Prof. T. Gurevich serves as a consultant to Abbvie. She received honoraria (from Abbvie and Novartis) and grants (from National Parkinson 's disease Foundation). She supports for the participation in scientific meetings from Teva, Medison, Abbvie, Allergan, Intecpharma, Megapharm, Novartis and Neuroderm.

Dr. B.El-Ad- reports no disclosures.

Ms. J. Tzamir - reports no disclosures.

Ms. Beatriz Hemo, - reports no disclosures.

Prof. C. Peretz-reports no disclosures.

*APPENDIX 1*

**A**: Generic drug list for tracing PD patients among Maccabi members

| ATC group name | ATC code | Generic name | Mechanism of action |
|---|---|---|---|
| Dopa & dopa derivatives | N04B A 02 | Levodopa + carbidopa tab (group 1) Levodopa + carbidopa gel (group1) Levodopa + benserazide caps (group 1) | Dopamine precursor + inhibitor of dopa decarboxylase |
| | N04B A 03 | Levodopa + carbidopa + entacapone tab (group 1) | Dopamine precursor + peripheral dopa decarboxylase inhibitor + COMT inhibitor |
| Adamantane derivatives | N04B B 01 | Amantadine(group 5) | Dopaminergic, anti-viral |
| Dopamine agonists | N04B C 01 | Bromocriptine (group 3) | Dopamine agonist |
| | N04B C 02 | Pergolide (group 2) | Dopamine agonist |
| | N04B C 04 | Ropinirol (group 2) | Dopamine agonist |
| | N04B C 05 | Pramipexole (group 2) | Dopamine agonist |
| | N04B C 06 | Cabergoline (group 3) | Dopamine agonist |
| | N04B C 07 | Apomorphine (group 7) | Dopamine agonist |
| | N04B C 09 | Rotigotine (group 2) | Dopamine agonist |
| | N04B C 10 | Lisuride (group 2) | Dopamine agonist |
| MAO B inhibitors | N04B D 01 | Selegiline (group 4) | MAO B inhibitor |
| | N04B D 02 | Rasagiline (group 4) | MAO B inhibitor |
| Other dopaminergic agents | N04B X 01 | Tolcapone (group 6) | COMT inhibitor |
| | N04B X 02 | Entacapone (group 6) | COMT inhibitor |

**B:** Algorithm for identifying PD cases and assigning them to accuracy levels (definite, probable and possible) based on drug purchase data

| Definitions | |
|---|---|
| Censoring | Death, transfer out of Maccabi Healthcare Services (MHS) or end of the study (December 31st, 2012), whichever occurred first. |
| Follow-up period (F-UP) | Time from 1st purchase to censoring. |
| Observation segment | The FUP is divided into consecutive observation segments of 12 months each, and the last observation segment with the residual number of months. |
| Purchase month | A month in which at least one purchase was made. |
| Final purchase gap | Time from last purchase to censoring |
| Examined interval | The examined interval is the period upon which most criteria are applied. For subjects with FUP ≥ 3 years (long FUP), the examined interval is a period of 3 consecutive, 12-months long observation segments, in which the purchase intensity (sum of purchase months of drug groups 1–6) was highest (see appendix 1 for a list of drugs and groups). For subjects with FUP < 3 years (short FUP), the examined interval is their full FUP. |
| Lag to 1st purchase | Time from the later between the start of the study (January 1st, 1998) and the start date of membership in MHS to 1st purchase. It is assumed that a lag to 1st purchase ≥ 1 year implies an actual 1st purchase, while a lag < 1 year suggests that drug purchases may have occurred prior to the 1st purchase recorded in the data employed in the study (i.e., before the study began or before the subject joined MHS). |

**C:** Full-detail algorithm. Terms defined above (section A) are *italicized* in the algorithm table below:

| | | | |
|---|---|---|---|
| 1 | minimum 1 purchase during the study period 1.1.1998–31.12.2012 | no→ | exclusion |
| | **yes↓** | | |
| **2** | 20 ≤ age at 1st purchase < 85 | **no→** | exclusion |
| | **yes↓** | | |
| 3 | minimum 3 purchase months within FUP | no→ | exclusion |
| | **yes↓** | | |
| **4** | purchases of GROUP 3 only (bromocriptine/cabergolin = dostinex) **OR** purchases of GROUP 5 only (amantadine) | **yes→** | exclusion |
| | **no↓** | | |
| **5** | *FUP* ≥ 3 years | **no→** | go to step 15 algorithm for *FUP* < 3 years |
| | **yes↓** | | |
| * * * * * * * * * | **algorithm for subjects with *FUP* ≥ 3 years** * * * * * * * * | | |
| **6** | at least 1 *observation segment* with a minimum of 3 *purchase months* | **no→** | exclusion |
| | **yes↓** | | |
| **7** | at least 1 *purchase month* of GROUP 7 (apomorphine) | **yes→** | certainty level: definite |
| | **no↓** | | |
| **Note: As of step 8, all criteria are applied to the *examined interval*** | | | |
| **8** | during the *examined interval*, at least 24 *purchase months* of GROUP 1, 2 or 4 | **yes→** | certainty level: definite |
| | **no↓** | | |
| | if age at 1st purchase < **75**: during at least 1 of the *observation segments* within the *examined interval*, minimum 9 *purchase months* of GROUP 1, 2 or 4 | **yes→** | certainty level: definite |
| **9** | if age at 1st purchase ≥**75**: during at least 1 of the *observation segments* within the *examined interval*, minimum 9 *purchase months* of GROUP 1 or 2; **OR** during any 2 of the *observation segments* within the *examined interval*, minimum 18 *purchase months* of GROUP 4 | **yes→** | certainty level: definite |
| | **no↓** | | |
| **10** | during at least 1 of the *observation segments* within the *examined interval*, minimum 6 simultaneous *purchase months* of drugs of 2 groups or more, any combination excluding (4 + 5) | **yes→** | go to step 13 |
| | **no↓** | | |
| **11** | if age at 1st purchase < **75**: during at least 1 of the *observation segments* within the *examined interval*, minimum 6 *purchase months* of GROUP 1, 2 or 4 | | |
| | **no↓** | | |
| **12** | during at least 1 of the *observation segments* within the *examined interval*, minimum 6 simultaneous *purchase months* of drugs of GROUP (4 + 5), **OR** minimum 3 simultaneous *purchase months* of drugs of 2 groups or more, any combination excluding (4 + 5)<br>if age at 1st purchase ≥**75**: during at least 1 of the *observation segments* within the *examined interval*, minimum 6 *purchase months* of GROUP 1 or 2, or 9 *purchase months* of GROUP 4; **OR** during any 2 of the *observation segments* within the *examined interval*, minimum 16 *purchase months* of GROUP 4 | **no→** | go to step 14 |
| | **yes↓** | | |
| **13** | *final purchase gap* < 365 days | **yes→** | certainty level: probable |
| | **no↓** | | |
| **14** | remaining subjects | **yes→** | certainty level: possible |
| **End** | | | |
| * * * * * * * * * | algorithm for subjects with *FUP* < 3 years * * * * * * * * | | |
| **15** | age at 1st purchase ≤ 65; **OR** *lag to* 1st *purchase* ≥ 1 year and deceased during the study period | **yes→** | go to step 6 |
| | **no↓** | | |
| **16** | at least 1 *observation segment* with a minimum of 3 *purchase months,* **OR** during any 2 *observation segments* within the *examined interval*, minimum 4 *purchase months*, **OR** – for subjects with *FUP* < 2 years – minimum 3 *purchase months* within the entire *FUP* | **no→** | exclusion |
| | **yes↓** | | |
| **17** | *lag to* 1st *purchase* < 1 year and deceased during the study period | **yes→** | go to step 7 |
| | **no↓** | | |
| **18** | at least 1 *purchase month* of GROUP 7 (apomorphine) | **yes→** | certainty level: definite |
| | **no↓** | | |
| **19** | During the *examined interval*, at least 24 *purchase months* of GROUP 1, 2 or 4 | **yes→** | certainty level: definite |
| | **no↓** | | |
| **20** | during at least 1 of the *observation segments* within the *examined interval*, minimum 6 *purchase months* of GROUP 1, 2 or 4 | **yes→** | certainty level: definite |
| | **no↓** | | |
| **21** | during at least 1 of the *observation segments* within the *examined interval*, minimum 6 simultaneous *purchase months* of drugs of 2 groups or more, any combination excluding (4 + 5), OR minimum 3 simultaneous *purchase months* of drugs of 2 groups or more, any combination of GROUP 1, 2, 4 and 6 | **yes→** | certainty level: definite |
| | **no↓** | | |
| **22** | during at least 1 of the *observation segments* within the *examined interval*, minimum 6 simultaneous *purchase months* of drugs of GR (4 + 5), **OR** minimum 3 simultaneous *purchase months* of drugs of 2 groups or more, any combination excluding (4 + 5) | **yes→** | certainty level: probable |
| | **no↓** | | |

| | | | |
|---|---|---|---|
| **23** | *final purchase gap* < 365 days | **no→** | go to step 25 |
| | **yes↓** | | |
| **24** | at least 1 *observation segment* with a minimum of 3 *purchase months* of GROUP 1, 2, or 4 | **yes→** | certainty level: probable |
| | **no↓** | | |
| **25** | remaining subjects | **yes→** | certainty level: possible |
| | **End** | | |

**PD case definition**: The algorithm will assign each defined PD case with one of three levels of plausibility – high, medium, low – based on a matrix of (A) age at first purchase, (B) specific drug type, (C) duration and density of purchases and (D) individual follow-up period criteria. The plausibility level will reflect the relative likelihood that a PD case defined in the study is indeed a true PD case.

(A) Age at first purchase: Patients whose age at first purchase was under 20 years will be excluded to avoid juvenile PD cases, which generally occur on clear genetic grounds. Patients who were 85-year-old or above at first purchase will be excluded too to avoid elderly cases that are most often miss-diagnosed. This is since PD motor onset is most probably before the age of 75, to a lesser extent in the following decade and rather improbable over 85. For the included patients (range 20–85), age at first purchase will be accounted for in combination with specific drug treatments with regard to the plausibility of PD.

(B) Drug type: The tracers are based on a list of 17 generic antiparkinsonian drugs, categorized as dopaminergic agents (ATC code N04B) and available in Israel during the study period (see appendix 1). These are incorporated in the algorithm divided into sub-groups by the mechanism of action and/or type of clinical use. E.g., subjects on single-drug therapy who use levodopa or dopamine agonists are more likely to be PD cases than those using amantadine. Therefore, if all other parameters are equivalent, the former would be assigned a higher plausibility level.

(C) Duration and density of purchases: Length of the period in which purchases were made will be accounted for, including the intensity of purchases. As PD is chronic and progressive, the longer the purchase period and the higher the density, the higher the likelihood of a true PD case.

(D) (Follow-up criteria: Due to the course of the disease, usually three years of follow-up in a specialist clinic are sufficient to establish a most probable diagnosis of PD. A person followed-up for three years or more since his first purchase, who has purchased APD rarely and sporadically, is likely not a true PD case. On the other hand, a patient that has begun purchasing APD only one year before the study period ended and presents a rather consistent (even if not high-intensity) pattern of purchases, may well be a true case. These factors will be considered in the designation of plausibility levels.

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