npj | parkinson's disease

Review

Published in partnership with the Parkinson's Foundation

https://doi.org/10.1038/s41531-025-01146-1

# "Recommendations for clinical study protocols for immune and inflammatory profiling in Parkinson's disease"



Laura Muñoz-Delgado[1,2] ✉, Caroline H. Williams-Gray[3], Gaetan Garraux[4], Diego Clemente[2,5,6], Özgür Öztop Çakmak[7], Julia C. Greenland[3], Inês Figueira[8,9], Isabel Machín-Díaz[2,5,6], Elenamaria Pirovano[10], Alessia di Flora[10], Clotilde Tournerie[3], Stamatia Papoutsopoulou[11], Sibel Ertan[7], Pablo Mir[1,2,12] & Cristoforo Comi[13]

Growing evidence supports the importance of immune processes in Parkinson's disease (PD). However, there is a need to improve the quality of observational clinical studies investigating the role of immunity in PD. In this context, an expert panel from the COST Action IMMUPARKNET (CA21117) aimed to develop guidance recommendations for conducting optimal immune profiling in PD. Firstly, criteria for inclusion and exclusion of participants, clinical data collection and participant stratification have been considered. Secondly, brain imaging of neuroinflammation has been reviewed. Finally, this review discusses sample collection, handling and storage of biological samples. In conclusion, this document aims to guide the scientific community in the optimal design of immune profiling studies in PD, so that we can generate robust and reliable data to advance our knowledge in this field.

Growing evidence supports the involvement of immunity in Parkinson's disease (PD), a neurodegenerative disorder characterised by loss of dopaminergic neurons in the substantia nigra. Recent studies suggest that both peripheral and central immune responses contribute to neuronal death in PD, potentially preceding clinical symptoms by several years[1]. In the past two decades, in vitro, ex vivo and in vivo studies have consistently shown the involvement of inflammatory responses mediated by microglia and astrocytes, which are linked to neurodegeneration and disease progression. Apart from that, more recent studies have demonstrated important changes in the peripheral immune profile within both the innate and adaptive compartments, particularly involving T lymphocytes and monocytes. However, changes in peripheral immune cell subsets are somewhat inconsistent across human studies, possibly due to differences in clinical phenotype and disease stage of patients with PD.

A better understanding of the mechanisms of immunity in PD could lead to the identification of targets for therapies to slow disease progression, as well as to the detection of new biomarkers to aid earlier diagnosis, allow immune-based stratification for clinical trials and monitoring of treatment response. Therefore, there is a need to improve the quality of observational studies devoted to elucidating the contribution of immunity in the development and progression of PD.

The clinical studies developed to date generally include small sample sizes and are widely heterogeneous in terms of inclusion and exclusion criteria, as well as in the clinical and genetic characteristics of patient cohorts. In addition, there is considerable heterogeneity in methods of sample collection and storage, which can have a substantial impact on biomarker analysis and results. The absence of clear guidelines for designing clinical studies specifically aimed at studying immunity may be delaying our understanding of its role in PD.

[1]Unidad de Trastornos del Movimiento, Servicio de Neurología, Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain. [2]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain. [3]Department of Clinical Neurosciences, University of Cambridge, Cambridge, CB2 0PY, UK. [4]MoVeRe group, GIGA-CRC Human Imaging & GIGA Neuroscience, University of Liege, Sart Tilman B35, 4000 Liège, Belgium. [5]Neuroimmune-Repair group, National Hospital for Paraplegics, SESCAM, Toledo, Spain. [6]Health Research Institute of Castilla-La Mancha (IDISCAM), Toledo, Spain. [7]Department of Neurology, School of Medecine, Koç University, Istanbul, Türkiye. [8]iNOVA4Health, NOVA Institute for Medical Systems Biology, NIMSB, Universidade Nova de Lisboa, 1099-085 Lisboa, Portugal. [9]iNOVA4Health, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade Nova de Lisboa, 1169-056 Lisboa, Portugal. [10]Center for Research in Medical Pharmacology, University of Insubria, Varese, Italy. [11]Department of Biochemistry and Biotechnology, School of Health Sciences, University of Thessaly, Larissa, Greece. [12]Departamento de Medicina, Facultad de Medicina, Universidad de Sevilla, Seville, Spain. [13]Department of Translational Medicine, Section of Neurology, University of Piemonte Orientale, Vercelli, Italy. ✉e-mail: lmunoz-ibis@us.es

In this context, to enhance the quality and consistency of observational studies exploring the role of peripheral immunity in PD, a panel of IMMUPARKNET experts, comprising leading researchers in PD and neuroimmunology, was convened. IMMUPARKNET (The role of IMMUnity in tackling PARKinson's disease through a Translational NETwork) is an Action supported by the European Cooperation in Science and Technology (COST), that supports a multidisciplinary research network to foster collaboration among scientists and clinicians studying immunity in PD[2]. The panel aimed to develop a guidance document for the clinical and biomedical research communities. This document provides recommendations for designing and conducting robust observational studies to investigate immunity's role in PD, to ensure reproducible and clinically translatable findings. It discusses criteria for participant selection and clinical characteristics tailored to study objectives. It also includes guidance on neuroimaging biomarkers of neuroinflammation. Additionally, it provides fundamental principles to support clinicians and researchers in developing effective sample collection and processing strategies for reliable immune biomarker analysis.

Given the scarcity of high-level evidence in the rapidly evolving field of immune system involvement in PD, a systematic review was not conducted, and recommendations were not graded. Instead, the expert panel performed a narrative review of the literature, which facilitated the development of informed and consensus-driven recommendations. The panel's collective expertise enabled a nuanced synthesis of fragmented evidence. Detailed narrative justifications, citing key studies and acknowledging evidence limitations, have been incorporated to ensure transparency and provide a robust and practical framework for researchers in the field.

## Criteria for inclusion and exclusion of participants

The first consideration is which participants, both PD patients and control subjects, should be included. Participant selection should be guided by the specific objectives of each study, while considering the relevance of clinically meaningful comparator groups. In large epidemiological studies, it is important to be as inclusive as possible in terms of participant recruitment to enable the exploration of diverse immune-related mechanisms and their associations with the presence and progression of PD. However, in studies focusing on detailed characterisation of inflammatory profiles in PD, it is important to carefully consider criteria for participant selection to minimise confounding factors.

To address both perspectives, we propose a two-step approach: (1) to characterise system dysregulation in PD versus controls and (2) to investigate interactions and common pathways with immune-related comorbidities.

For Step 1, we propose that initially core studies should be conducted, consisting of patients with PD and controls without immune-related comorbidities, such as cancer, inflammatory or infectious diseases. Such studies will enable detailed immune profiling to identify PD-specific immunological signatures, free from confounding factors. We recommend the following pragmatic inclusion and exclusion criteria for defining core study cohorts, as outlined below and summarised in Table 1.

For Step 2, we propose extended studies including subjects with immune-related comorbidities. These will investigate whether these comorbidities share immune dysregulation pathways with PD or influence its pathogenesis and progression.

### Inclusion criteria

There must be diagnostic confidence in the diagnosis of PD. Currently, the most widely accepted criteria are the Movement Disorder Society (MDS) Diagnostic Criteria for PD[3], which requires the presence of Parkinsonism, together with supportive features balanced against 'red flags'. However, these criteria are not well suited to recently diagnosed cases in whom supportive criteria and some 'red flags' may not have had time to evolve, and so for early/de novo cohorts, the revised MDS criteria for clinically established early PD may be more suitable[4]. There is an ongoing debate about the need for additional biomarker testing, such as the alpha synuclein seeding assay, dopamine transporter imaging, and genetic testing to provide biological confirmation of the diagnosis[5,6]. Whilst these newly proposed classifications of PD may strengthen confidence in the diagnosis, they still require further validation and may limit recruitment in centres where such testing is not readily available.

Prodromal PD cases may be interesting to include as they can help us to understand early disease mechanisms and to identify predictive biomarkers. In this line, accumulating evidence has shown an immune activation in patients with rapid eye movement (REM) sleep behaviour disorder (RBD)[7,8] known to be at high risk of developing PD. A diagnosis of prodromal PD can be made by employing the MDS criteria[9]. Furthermore, including carriers of variants in PD-associated genes who are considered at risk of developing PD, and comparing their immune profiles between those who eventually develop the disease and those who do not, may provide valuable insights into both causative and potentially protective immune responses[10–12].

In terms of the selection of control participants, matching to the age range and sex of the PD population is critical, given the well-established impact of ageing and sex on the immune system[13,14]. Furthermore, sex-dependent changes in the immune system have been reported in PD[15,16].

### Exclusion criteria

For studies investigating the relationship between the immune system and PD, it is important to consider the impact of other clinical factors which may confound any alteration in the immune profile. A wide range of comorbidities and medications may influence inflammatory status. As mentioned at the beginning of this section, a balance between designing a robust study which explores PD-relevant immune changes and obtaining a representative sample of patients should be considered. Consistent with the two-step approach, the following exclusion criteria are recommended for core studies in the first step.

Firstly, we would recommend excluding any participants with inflammatory or autoimmune disease[17]. For conditions with an immune component but which are characterised by intermittent flares (for example gout[18]), an alternative approach would be to allow recruitment of these participants, ensuring this comorbidity is inactive at the time of assessment. Participants with either acute or chronic infection should be excluded[19]. Both should ideally be assessed with a clinical review. However, a blood test for C-reactive protein can also be a useful screen for an acute condition, with a cut-off of >10 mg/L being most adopted as an exclusion criterion[20]. Exclusion of patients with active solid organ malignancy is warranted[21]. In the case of haematological malignancy, not only active but also previous malignancy may be considered, given the possibility of residual immune perturbation[22,23]. Recent major surgery or trauma should also be an exclusion criterion[24,25], with the timeframe being a matter of clinical judgement, but our consensus opinion being at least 6 weeks. Diabetes mellitus is associated with changes in both innate and adaptive immunity[26]. Given that this is most pronounced in hyperglycaemic states, well-controlled diabetes need not necessarily be a reason for exclusion.

Participants currently taking immunosuppressant medication should be excluded. The majority of immunosuppressants have long-lasting effects[27]. Therefore, either a sufficient wash-out period after their discontinuation must be an exclusion criterion (for example 1 year in the case of broad-spectrum agents such as azathioprine or methotrexate), or it may be appropriate to exclude participants with any prior use, particularly where high potency agents such as cyclophosphamide, rituximab or alemtuzumab have been administered, which have long-lasting effects on the immune system. Steroids could potentially be treated differently, given their shorter immunosuppressive effect, with one study showing a change in immunoglobulin levels lasting 4 weeks following a short course[28]. Therefore, a reasonable criterion would be no oral steroid use in the past 3 months. There is some evidence of a systemic effect of inhaled, topical or nasal steroids[29,30], and hence it may be appropriate to exclude current users of these therapies.

Anti-inflammatory medication is commonly used in this age group, and therefore, there are challenges in adopting this as an exclusion criterion

**Table 1 | Recommended pragmatic inclusion and exclusion criteria for recruitment to the core studies for detailed characterisation of immune and inflammatory profiles in Parkinson's disease (PD) and/or prodromal PD compared to controls**

| | |
|---|---|
| **Essential inclusion criteria** | |
| | MDS criteria for PD[a] Clinically established PD, Clinically probable PD |
| **OR** | MDS criteria for early PD (<5 years)[b] |
| **OR** | MDS criteria for prodromal PD[c,d] (cut-off for likelihood for prodromal PD defined as ≥80% certainty) |
| **OR** | Control status defined by the lack of neurological disease on clinical screening |
| **Essential exclusion criteria** | |
| | Primary inflammatory or autoimmune disorders |
| | Active cancer or any prior history of haematological malignancy |
| | Recent major surgery or trauma (<6 weeks) |
| | Acute infection (clinical assessment ± C-reactive protein >10 mg/L) |
| | Chronic infection (e.g. HIV, hepatitis) |
| | Immunosuppressant medications (long-acting immunomodulatory agents within the past 12 months; oral steroids within the past 3 months) |
| | Anti-inflammatory medication (other than low-dose aspirin ≤75 mg) |
| | Vaccination (<4 weeks) |

[a]Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591-601.
[b]Berg D, Adler CH, Bloem BR, et al. Movement Disorder Society Criteria for Clinically Established Early Parkinson's Disease. Mov Disord. 2018;33(10):1643-1646.
[c]Heinzel S, Berg D, Gasser T, et al. Update of the MDS Research Criteria for Prodromal Parkinson's Disease. Mov Disord. 2019;34(10):1464-1470.
[d]Berg D, Postuma RB, Adler CH, et al. MDS Research Criteria for Prodromal Parkinson's Disease. Mov Disord. 2015;30(12):1600-11.

for studies of immunity in PD, given the large number of patients who would be rendered ineligible. We suggest not excluding patients based on the use of low-dose aspirin (75 mg). However, we would suggest that participants should be excluded if they have used aspirin at a dose of over 75 mg or non-steroidal inflammatory agents within the previous 2 weeks[31,32].

Vaccinations are known to lead to an altered immune profile and are frequently relevant in this age group. Considering that the primary response to a vaccination typically occurs over 2 weeks[33], it would seem reasonable to wait at least four weeks post-vaccination until recruitment into the study.

For control participants, clinical screening to exclude neurological disease, in addition to the factors discussed above, is essential. This recommendation is intended to minimise false positives and false negatives among control participants. However, future investigations could also incorporate other neurodegenerative conditions (e.g. other synucleinopathies, tauopathies, and amyloid-beta pathologies) as controls in comparator groups to determine whether the observed immune profiles are unique to PD or shared across neurodegenerative conditions[34–36]. This strategy will enhance specificity and help to distinguish PD-related immune mechanisms from those in related pathologies.

Exclusion of people with a first-degree relative with PD as controls is also recommended, given the possibility of carriage of a PD risk gene, which may be associated with an altered immunophenotype[11,12].

In the second step of our recommended approach, the focus shifts to exploring interactions and shared pathways with immune-related comorbidities. Accordingly, both PD patients and control participants with such conditions may be included under more flexible exclusion criteria. Comparing PD and control groups with inflammatory or autoimmune disorders may provide valuable insights into common and divergent immunological mechanisms between PD and other chronic inflammatory conditions[37,38]. This strategy ultimately enhances the specificity and interpretability of the findings.

## Study design

Clinical studies in PD are usually hampered by a long duration and slow progressive course of the disease, the clinical heterogeneity and the variability of symptoms and signs over the day related to the time of medication and polytherapy. In this regard, the approach to a successful clinical study design is challenging and requires consideration of several aspects, including whether to adopt a cross-sectional or longitudinal timeline, selection of the most appropriate clinical measures, and the consideration of stratification into subgroups for analysis.

### Cross-sectional versus longitudinal design

Cross-sectional studies are easier to perform, faster and less expensive than longitudinal studies. In a field that is largely underexplored, cross-sectional studies are conducted with the main aim of generating research hypotheses. For example, in a cross-sectional study, peripheral blood T cell phenotypes differed significantly between cognitively intact and impaired PD patients[39]. Such findings may suggest a relationship between the peripheral immune profile and cognitive evolution of PD, but a cause-and-effect relationship can only be demonstrated through a longitudinal study, in which both the immunophenotype and cognitive status are repeatedly recorded over time, starting from disease onset, when patients were still drug-naive. Such a study design would obviously require a long observation, due to the relatively low rate of PD patients converting to dementia in a short timeframe (about 10% per year)[40], but this issue could be mitigated by including neuroimaging data along with fluid biomarkers associated with neuronal damage as surrogate markers. Longitudinal studies are also essential to address the question of when the immune response is most prominent in PD and how it changes with disease course, which is important to guide the timing of future immune-based therapeutic interventions. Studies which follow patients from the prodromal stage through their disease course are needed to further investigate this.

However, researchers often face several challenges when performing longitudinal studies[41]. Patient retention is one of the most significant issues, particularly in neurodegenerative diseases like PD, where the progression of symptoms can affect patient participation[42,43]. To address this, flexible study designs that offer adaptable scheduling for visits, as well as early and clear communication about the long-term nature of the study and its potential benefits, can help encourage continued participation. Additionally, providing incentives such as regular updates on the study's progress or personalised health reports can encourage continued participation and a sense of involvement. By maintaining regular follow-ups and personalised communication, participants are more likely to feel like active contributors rather than passive subjects. Another key challenge in longitudinal studies is the cost associated with repeated immune monitoring, which requires multiple sample collections, clinical visits, sample storage and other logistical components over time. Lastly, ensuring the reliability of biomarker

assays over time is crucial. Variability between timepoints can be enhanced by running batch analyses of samples collected across multiple visits, in order to minimise longitudinal drift in assay performance. To facilitate this, it is essential to implement standardised and validated protocols for sample collection, processing, and storage to minimise sample degradation. Relevant recommendations on this are included in the following section of this manuscript.

### Clinical data and patient stratification

Given that conducting clinical studies can be limited by the clinical setting (lack of time and clinicians and/or resource facilities), two levels of clinical data collection are suggested. The first covers essential and minimal data that should be collected to allow basic clinical characterisation, and the second provides a more comprehensive assessment for specialised centres with more research resources. A schematic summary is displayed in Table 2.

**Demographic data**. The age and sex of the participants are essential to allow careful matching of PD and control cohorts and may need to be considered as stratification variables. Age is well known to influence the immune system[13], and age of PD onset may also be important, as some studies have indicated that immune factors have a more prominent role in late-onset PD, compared to early-onset[44]. Subgrouping of participants by sex within the study design should be considered based on previous findings of sex-dependent immune changes in PD[15,16,45]. In addition, ethnic differences in immune response have been reported in several studies[46,47], therefore, patient ethnicity should be considered when studying the role of immunity in PD.

**Genetic status**. Genetic factors must be considered in the complex aetiopathogenesis of PD and may warrant consideration in the selection of participants for studies of immunity. Several genes have been associated with PD, including variants in genes responsible for Mendelian forms of the disease (such as leucine-rich repeat kinase 2, *LRRK2*) and genetic risk factors (such as heterozygous variants in glucocerebrosidase, *GBA*). These genes encode proteins involved in specific cellular pathways related to immunity and inflammation[48,49] and patients with variants in PD-associated genes have shown different alterations in the peripheral immune profile[50,51]. Interestingly, different inflammatory pathogenic mechanisms involved among the genetic and sporadic forms could also be responsible for inconsistent results on immunity in PD reported to date. In addition, patients with variants in PD-associated genes have distinct clinical features and disease progression, which at the same time have been linked to different inflammatory profiles[49,50]. Therefore, it is highly recommended to consider the genetic background, at least of the most frequent genes associated with PD, when designing studies investigating immunity in PD.

**Comorbidities**. Common age-related comorbidities such as metabolic syndrome, cardiovascular disease, hypertension, diabetes and dyslipidemia are recommended to be recorded. These conditions can indirectly affect immune system activation and may interact with immune dysregulation in neurodegenerative diseases, including PD[52–54]. Integrating these factors into study design and data analysis will enable researchers to determine whether immune dysregulation in PD is influenced by or independent of these prevalent comorbidities in the second-step approach.

**Environmental and lifestyle factors**. To gain a more comprehensive understanding of environmental contributors to the neuroinflammatory processes implicated in PD, it is recommended to capture detailed

**Table 2 | Two-level clinical data collection is recommended for clinical studies investigating the contribution of immunity to the development and progression of Parkinson's disease (PD)**

| | Level 1 – essential | Level 2 - extended |
|---|---|---|
| Demographics | Age | |
| | Sex | |
| | Age at onset | |
| | Disease duration | |
| Genetic status | - | Genotyping for PD risk variants |
| Medication | Dopaminergic treatment and Levodopa equivalent daily dose (LEDD)[a]<br>Anti-inflammatory or immunomodulatory drugs | Detailed medication history |
| Motor status | Hoehn & Yahr stage[b] | MDS-Unified Parkinson's Disease Rating Scale[c] |
| Cognitive status | Global test of cognitive function (e.g. Montreal Cognitive Assessment, Mattis Dementia Rating Scale, Parkinson's disease-Cognitive Rating Scale) | Specific-domain neuropsychological battery |
| Non-motor symptoms | History of depression/anxiety | Non-motor symptom scale (NMSS)<br>Depression/anxiety scale (e.g. Patient Health Questionnaire-9, Hospital Anxiety and Depression Scale, Geriatric Depression Scale)<br>Evaluation of gut symptoms (e.g. Gastrointestinal Dysfunction Scale for Parkinson's Disease)<br>Olfactory evaluation (e.g. UPSIT or Sniffin' Stick Test) |
| Comorbidities | | Metabolic disorders<br>Cardiovascular disease<br>Gastrointestinal disorders |
| Environmental and lifestyle factors | | Geographical and occupational histories<br>Trauma<br>Vaccination records<br>Diet, exercise, and sleep |

[a]Jost ST, Kaldenbach MA, Antonini A, et al. International Parkinson and Movement Disorders Society Non-Motor Parkinson Disease Study Group. Levodopa Dose Equivalency in Parkinson's Disease: Updated Systematic Review and Proposals. Mov Disord 2023;38(7):1236-1252.

[b]Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17(5):427-42.

[c]Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. Mov Disord 2008;23(15):2129-70.

lifelong exposure histories along with relevant lifestyle information[55]. First, detailed records of geographical and occupational histories, as well as incidents of trauma, should be collected. Exposure to pesticides, insecticides or heavy metals warrants consideration during patient selection and stratification, as there is accumulating evidence from experimental and epidemiological studies suggesting their contribution to neuroinflammation in PD[56]. Additionally, vaccination records, including those not administered, as well as histories of past infections, should be documented[57], and serological studies may be conducted to provide a more detailed history of exposure of common viruses.

On the other side, lifestyle factors such as diet[58], exercise[59] and sleep[60] have been shown to modulate immune function and could significantly impact the immune profiles of PD patients. Stratifying participants based on their habits can help identify how lifestyle influences immune responses in PD.

**Dopaminergic treatment**. Concurrent dopaminergic treatment should also be considered. Emerging evidence has shown dopamine as a key transmitter between the nervous and the immune systems as well as a mediator produced and released by immune cells themselves[61]. Dopamine receptors are widely expressed on immune cells, which can mediate stimulatory or inhibitory responses, and exogenous dopamine has been shown to have a range of effects on different immune populations in vitro[62,63].

Given this evidence, treatment status must be carefully considered in study design. Distinguishing—and where possible, comparing—treatment-naïve and treated PD patients is essential when studying immune profiles. While some studies take the approach of only recruiting drug-naïve patients, this approach may not always be feasible depending on local clinical practice and timing of patient referrals into research programmes. Alternatively, dopaminergic medication should be recorded at the time of sampling using a standardised method, such as calculation of a levodopa equivalent daily dose (LEDD)[64], so that this can be controlled for within subsequent analyses.

**Disease symptoms and signs**. PD clinical heterogeneity may be mediated by different underlying pathogenic mechanisms, including immunity. For example, a peripheral proinflammatory profile has been associated with a more severe motor and cognitive phenotype and faster progression[39,65–70]. Thus, a more detailed stratification based on clinical phenotype should be considered.

- Disease duration
  Disease duration/stage is an important consideration because there is evidence to suggest that immune activation may play a more prominent role in the early stages of the disease[71,72]. Failure to stratify based on disease duration is likely to introduce heterogeneity into immune profiling and lead to difficulty interpreting findings. It should be noted that disease duration is typically measured from the date that the diagnosis was given, but with the caveat that the underlying PD pathology is well established by this point. Duration from symptom onset is an alternative approach, although it can be affected by recollection bias and is problematic in studies recruiting later-stage patients.
- Motor function
  A wide variety of rating instruments are available for assessing motor function, including Hoehn and Yahr (HY) staging and the MDS-Unified Parkinson's disease Rating Scale (UPDRS), and specific scales to report motor complications, such as the freezing of gait questionnaire[73]. The need for a comprehensive motor evaluation needs to be balanced against time constraints in clinical settings. We consider that the HY scale is essential for motor severity stratification. However, it is widely acknowledged that each stage on the scale can encompass considerable heterogeneity[74]. Therefore, the MDS-UPDRS[75] is recommended as a comprehensive tool for motor evaluation in research settings.
- Non-motor symptoms
  Immune changes and neuroinflammation have been strongly implicated in cognitive impairment and dementia in PD[39,69,70,76]. For classifying patients according to their cognitive status, the current accepted diagnostic criteria for mild cognitive impairment (PD-MCI)[77] and dementia (PD-D)[78] should be followed. Cognitive scales that measure global cognitive performance can be useful tools for screening and can provide reasonable coverage of multiple cognitive domains. For this purpose, the scales most recommended by the Movement Disorders Society are the Mattis Dementia Rating Scale, the Montreal Cognitive Assessment (MoCA), and the Parkinson's Disease-Cognitive Rating Scale (PD-CRS)[79]. A more comprehensive neuropsychological assessment, consisting of tests within each cognitive domain, would allow a more detailed exploration of the association between immune changes and cognition, although these tests are time-consuming, require specialised training, and may not be feasible in a busy clinical practice.

To date, little is known about the link between the immune system and other non-motor symptoms in PD. However, there is strong evidence that depression and anxiety, which are common features of PD, are linked to immune dysfunction[80], and it is therefore important to consider depression status when evaluating immune changes in PD, which can be done using a short patient-completed questionnaire (see Table 2). Assessment of gut symptoms should also be considered, given the accumulating evidence that gut dysfunction and associated microbial changes cause a local inflammatory response, which may influence PD progression via a gut-immune-brain axis[81,82]. Gut symptoms can be quantified using the Gastrointestinal Dysfunction Scale for Parkinson's disease (GIDS-PD), which also captures information on diet[83]. Olfactory dysfunction is a clinically relevant and early non-motor feature of PD, which has been associated with both immune mechanisms and α-synuclein pathology within the olfactory bulb[84]. Standardised and validated approaches for olfactory testing include the University of Pennsylvania Smell Identification Test (UPSIT)[85] and the Sniffin' Sticks test[86]. A scale encompassing a broader range of non-motor symptoms, such as the NMSS, should also be considered in research settings[87].

## Brain imaging of biomarkers of neuroinflammation

Neuroinflammation can be assessed in vivo using imaging techniques, which have the advantage of providing spatially informative and potentially quantitative measurements in the brain.

### Positron emission tomography (PET) imaging

PET imaging is regarded as the gold standard to depict the neuroimmune endophenotype of many brain conditions. Several PET radiotracers of candidate biomarkers of neuroinflammation have been developed. Initially identified as the peripheral benzodiazepine receptor, the translocator protein (TSPO) remains the most extensively studied molecular target to assess neuroinflammation using PET in humans[88–90]. The central nervous system (CNS) normally expresses TSPO at low levels, making it notable when there is significant upregulation associated with a response to injury and disease.

To date, several dozen TSPO radioligands have undergone preclinical or clinical evaluation. The first PET radiotracer used in PD is the isoquinoline derivative (R)-[$^{11}$C]PK11195. However, its intrinsic imaging limitations led to the development of 'second-generation' TSPO radioligands aimed at providing a better signal-to-noise ratio. An important limitation regarding the clinical application of these second-generation radiotracers is their universal sensitivity to the rs6971 single-nucleotide polymorphism of the TSPO gene on chromosome 22q13.2, so that study participants may have a high-affinity, mixed-affinity or low-affinity binder phenotype, depending on their particular TSPO gene polymorphism and TSPO binding features. Individuals with two copies of the rare allele (i.e. low-affinity binders) bind these radioligands with a lower affinity than people with two copies of the major allele (i.e. high-affinity binders), and people who are heterozygous for this allele (i.e. mixed-affinity binders)

express both high-affinity and low-affinity binding sites in similar proportions. The allelic abundance in humans is ~5:2 in favour of the high-affinity binding allele (HAB), such that 10% of humans will show little or no TSPO-specific binding, and approximately 40% will show intermediate binding. Thus, individuals with similar TSPO expression but different genotypes will produce different PET signals using second-generation TSPO tracers, while the prototypic tracer [11 C]-(R)-PK11195 is indifferent to this allelic status. This obstacle could be partly addressed by performing TSPO genotyping prior to PET imaging to exclude low-affinity binders[89].

In PD patients, cross-sectional studies using first- and second-generation PET radioligands have mainly shown increased TSPO binding, predominantly in the midbrain, compared with matched control participants[91]. Longitudinal assessment of TSPO binding is lacking in PD. Nevertheless, PET studies consistently showed that regional increases in TSPO binding can be detected early in the disease course[76,92,93], including in de novo patients[94], individuals in the premotor phase of the disease[95] and some asymptomatic gene mutation carriers at risk for PD[96]. These results provide evidence suggesting that it is unlikely to be confounded by dopaminergic treatment, which can interfere with both the inflammation response[97] and TSPO binding quantification[98].

Interestingly, TSPO is just one marker of the inflammatory response, and new molecular targets to assess neuroinflammation using molecular PET imaging are currently being developed, targeting both microglia and astrocytes at different activity states[99]. None have been extensively investigated in PD.

The drawbacks of molecular PET imaging include the low availability of radiochemistry facilities, ionising radiation exposure, which limits its use in vulnerable populations and longitudinal studies, and low spatial resolution, making it unsuitable to image small structures satisfactorily.

### Magnetic resonance imaging (MRI)

In the future, these drawbacks could be overcome by molecular MRI, but the latter is still in infancy. In the meantime, an emerging application of MRI in the field of neuroinflammation is to investigate biomarkers of brain—border immune niches, which are believed to contribute to the development of neurodegenerative disorders[100]. However, the exact pathophysiological mechanisms underlying many of these new biomarkers still need to be cross-validated in humans. Currently, the most well-established application of MRI is to allow anatomical co-localisation of molecular PET findings of neuroinflammation. A common approach consists of the acquisition of high-resolution T1-weighted MR images of the entire brain using a 3D magnetisation-prepared rapid gradient echo (MPRAGE) or an MP2RAGE sequence at ultra-high field strengths[101].

## Biological samples and immune biomarkers

Sample collection, handling and storage are crucial steps in the study of immune-related biomarkers, where a standardised approach is essential to ensure reliability. In this section, we will discuss basic principles that can be useful to clinicians and researchers to help determine appropriate sample collection and processing strategies to enable reliable immune biomarker analysis.

Immune biomarkers can include cell-based measures of phenotype and function, as well as measurement of inflammatory mediators (cytokines and chemokines). Alterations in the frequency of blood immune cell subsets, as well as functional changes, are well described in PD[1]. There is evidence for lower lymphocyte numbers in PD patients compared to controls, but there are conflicting results on specific T-cell and B-cell subpopulation dysregulations[102]. Changes in T cell and monocyte subsets have also been reported in the cerebrospinal fluid (CSF) in PD, although studies have been limited to date[103,104]. Notably, research on the role of neutrophils in PD is lacking, due to the standard approach of isolating peripheral blood mononuclear cells (PBMCs; comprising lymphoid and myeloid cells), which discards the polymorphonuclear cells (PMNCs, i.e. granulocytes). The observed discrepancies in immunophenotyping results across studies may be related not only to differences in the clinical phenotype of PD patients, but also to the methodology used in sample collection and analyses. Several key pre-analytical variables can affect cell quality, as discussed below in relation to specific sample types.

Cytokines and chemokines have been reported to be increased in serum, plasma and CSF in PD, and some studies have shown links with disease stage and progression[68,102,105,106]. However, a wide disparity exists between studies in terms of which markers are altered. As for cell-based studies, discrepancies may relate to differences in methodology employed in sample collection, processing and storage. There is an intricate relationship between the clock gene system, the circadian rhythm, and the pathology underlying PD[107–109]. Circadian control of innate immune cell movement and pathogen response has been shown, together with day/night differences in the adaptive immune cell response[110]. Therefore, it is very important to keep the timing for biosample collection constant within studies. Correlation between blood and CSF cytokine levels has been shown to be poor, likely reflecting local cytokine production within the CNS, and suggesting that compartment-specific analyses are needed[111]. Other biological samples of interest to study inflammatory mediators include faecal samples and saliva.

Below, we address key considerations for the collection and processing of individual sample types and optimal biomarker analyses. Table 3 provides a summary of samples and biomarkers recommended for immune profiling studies in PD. While biospecimen selection should ultimately be guided by the specific research objectives, a two-level collection framework is proposed, considering both scientific value and feasibility. For example, CSF and functional assays offer particularly critical insights, but they have practical feasibility constraints that need to be considered. Table 4 and Fig. 1 further outline recommendations for sample collection, processing and storage.

### PBMCs

The analytical endpoint use of PBMCs can feed into two assay categories, with implications for the collection and processing method: (1) assays not requiring viable cells, such as nucleic acid (NA)-based genetic analysis, for which immediate stabilisation after isolation is critical to preserve NAs. Cells should be stabilised in an appropriate NA stabiliser and then stored frozen until NA extraction[112]. (2) assays requiring intact/viable cells, such as immunophenotyping and functional assays. These assays are sensitive to the conditions under which PBMCs are collected, processed, and stored[113].

- PBMC isolation methodology

  The choice of blood collection tubes is important when collecting blood for PBMC extraction. PBMCs are typically isolated from peripheral blood samples collected in tubes containing anticoagulants such as lithium heparin. Although ethylenediaminetetraacetic acid (EDTA) tubes provide higher PBMC isolation efficiency compared to heparin[114], citrate-based anticoagulants like acid citrate dextrose (ACD) and citrate phosphate dextrose (CPD) are beneficial in preserving cell functionality over longer storage periods[115].

  Common methods for PBMC isolation include density gradient centrifugation with Ficoll-Paque, and isolation with ready-to-use cell preparation tubes, such as CPT or Leucosep/Lymphoprep, which contain a density gradient medium that enables PBMC isolation while preserving cell integrity and viability[116]. CPT or Leucosep have been shown to be simple and fit-for-purpose for the isolation of high-quality immune cell subpopulations[117]. They have the advantages of ease of handling, timeliness, safety and reduced variability. However, manual isolation with EDTA tubes renders a higher cell yield than other anticoagulants[114]. It is highly recommended to test and document the type of anticoagulant and isolation method used for each study and maintain consistency within the same study.

  Delayed processing can adversely affect PBMC viability and function, so minimising time between collection and processing is crucial for ensuring quality[118,119]. Samples should ideally be processed within two hours at room temperature, followed by immediate cryopreservation. If this is not feasible, as a consensus, blood can be left for up to 8 h

**Table 3 | Two-level sample and biomarkers collection recommended for clinical studies investigating the contribution of immunity to the development and progression of Parkinson's disease (PD), considering both scientific value and feasibility**

| | Level 1 – essential | Level 2 - optional |
|---|---|---|
| Sample Collection | Serum | PMNCs |
| | Plasma | CSF |
| | PBMCs | Saliva |
| | | Urine |
| | | Faeces |
| Biomarkers | Immunophenotypes (immune cell subsets and inflammatory markers) | Functional immune assays |
| | | Proteomics |
| | | Transcriptomics |

(never >24 h) at room temperature on a shaker to prevent coagulation before processing. However, it should be noted that delayed processing or prior cold storage can selectively bias the survival of specific immune cell populations as well as compromise RNA integrity[120–122].

- Volume of blood
  The number of PBMCs required depends on the specific downstream application, i.e. flow cytometry (FACS), magnetic-activated cell sorting (MACS) or stimulation assays. Researchers should refer to specific assay protocols to determine the number of cells needed. Standard PBMC isolation using a Ficoll-Paque gradient technique typically yields 0.5–2 million PBMCs per millilitre, and hence the appropriate blood volume needed can range from 10 to 50 mL per assay[117].
- Fresh versus cryopreserved PBMCs
  The choice between fresh and frozen PBMCs will depend on several factors, such as cell type, cell functionality, the downstream assay and logistical and timing flexibility. In general terms, fresh PBMCs ensure maximum cell functionality and accurate immunophenotyping results[123–125]. Frozen storage is limited for genetic analysis, considering the instability of DNA after long-term storage of PBMCs[126–128], but also for the design of immune cell profiling studies[129,130].
  Despite these limitations, freezing PBMCs allows for more efficient bulk analyses, which is particularly important in longitudinal studies and multicentre projects. To minimise damage during freezing, PBMCs should be cryopreserved using a controlled-rate freezing process and stored in cryoprotective medium (10% DMSO in foetal calf serum) in liquid nitrogen[131–133]. Storage of PBMCs at −80 °C must be avoided since it increases their apoptosis rate and induces substantial genomic modifications[134,135]. Of note, to minimise inter-centre variability, a cell concentration greater than $6 \times 10^6$ PBMC/mL has been associated with improved viability[136].
- Quality control of PBMCs samples
  Finally, a systematic assessment of cell counting, cell viability and cell contamination (by dead cells, platelets and/or red blood cells (RBC)) is essential to improve sample purity, downstream data accuracy, as well as reliability and interpretability of immunological data. Cell counting can be done by using different standardised counting methods[137,138]. Systematic verification of cell viability can be carried out by using specific stains or fluorescent dyes to provide accurate discrimination of live and dead cells during flow cytometry analysis[137]. Platelet contamination can be minimised by washing and centrifugation, and identified in flow cytometry either by their smaller size during gating or by specific staining or markers[139–141]. Regarding RBC contamination, the main approaches include chemical lysis or the use of nucleic acid–binding dyes such as acridine orange and propidium iodide, as well as the application of specific markers for flow cytometry identification[138,140].

### PMNCs

There is a knowledge gap on the role of neutrophils on PD, mainly due to methodological issues[1].

Percoll gradient is the most commonly adopted strategy for PMNC isolation[142–144] and has been reported to achieve a slightly higher degree of purity compared to Ficoll[145]. Furthermore, isolation of PMNCs using a Percoll gradient or spontaneous sedimentation technique reduces PMNC priming, which is crucial when planning functional assays rather than immunophenotyping[143,146]. However, when the amount of blood is limited, PMNC and PBMC isolation can be done from the same sample using Leucosep™ tubes, with density gradient centrifugation using Ficoll, resulting in the formation of a layer of PBMCs and a layer of PMNCs with red blood cells. Isolation of PMNCs and PBMCs can then proceed in parallel[147]. Both cell types can be combined following isolation for the purposes of immunophenotyping.

### Serum and plasma

Serum and plasma are separated from whole blood through centrifugation at 1000–2000G for 10–15 min. For plasma, blood is collected into a tube containing anticoagulants, whereas serum is obtained from blood collected in a plain tube, which is left to clot for at least 15 min prior to centrifugation. Factors such as the use of anticoagulants, storage tube contamination with endotoxins, and delays in blood processing (centrifugation) can have a major impact on measured cytokine concentrations in both blood components. In terms of the choice of sample for analysis, plasma has been suggested to be a more sensitive matrix for detecting changes in low-abundance cytokines compared to serum[148]. Serum has been found to have higher levels of some cytokines than plasma, which may be due to the release of cytokines by activated platelets during the coagulation process[149]. On the other hand, prior to plasma separation from whole blood, leucocytes can secrete cytokines in vitro, thus altering cytokine levels; thus, it is important to minimise sample processing delays. The choice of anticoagulant in the blood collection tube may also influence cytokine levels in plasma[150–152]. Plasma collection with EDTA has been shown to produce the most consistent results for cytokine analyses and more closely resembles data obtained in serum[148].

Importantly, delays in blood processing can lead to changes in cytokine levels due to cellular release or degradation; therefore, it is advisable to process samples within 60 min of collection. Keeping samples on ice or at 4 °C during processing can help maintain stability. At room temperature, serum cytokines are more susceptible to delayed processing compared to plasma[153]. Since repeated freeze-thaw cycles can lead to cytokine degradation, it is recommended to aliquot samples into small volumes (e.g. 200 µL) to avoid multiple freeze-thaw cycles, and this point should be considered when planning sample collection. In addition, it is recommended to use low-binding tubes (polypropylene rather than polystyrene tubes) to minimise cytokine adsorption to the tube walls. Long-term storage should be at −80 °C to minimise protein degradation.

### CSF

CSF should be stored on ice as soon as possible post collection. It should be ideally processed by centrifugation (for 500–1500×*g* for 10–15 min at 4 °C) within 60 min to separate cells from supernatant[154,155]. The cell pellet should be used immediately for immunophenotyping using flow cytometry, as due to the low number of cells, the cell yield post freezing is generally insufficient for meaningful analysis[156]. The supernatant is stored at −80 °C for subsequent protein measurement, extracellular vesicle extraction, or cell-free RNA analysis[154,157]. Single-cell analyses require specialised processing and freezing conditions to maintain cellular integrity and transcriptomic profiles, as detailed in specific manuscripts[158,159].

### Faecal samples

Cytokine analysis in stool can potentially provide valuable insights into gastrointestinal inflammation and immune responses in the gut[160]. Moreover, it has been shown that there are correlations between cytokine levels

**Table 4 | Summary of recommendations for sample collection, processing, and storage**

| Step | Sample type | Key recommendations | Other recommendations |
|---|---|---|---|
| **Participant Preparation** | | | |
| | Blood | | Overnight fasting when appropriate. |
| | CSF | Adequate hydration. | |
| | Faecal | Instruct to avoid urine contamination; record bowel symptoms and laxative use. | |
| | Saliva | No food, drink, smoking, or brushing 1 h before; rinse mouth 10 min before; record oral health status. | |
| **Collection method** | | | |
| | Blood | Collect in appropriate tubes (EDTA, heparin, citrate, plain). | Tube choice affects downstream analyses |
| | PBMCs | Tubes containing anticoagulants are recommended: heparin standard, EDTA higher yield, Citrate (ACD or CPD) preserves functionality; collect 10–50 mL | Standard Ficoll isolation yields 0.5–2 million PBMCs/mL; adjust blood volume according to assay needs. |
| | Serum | Collect blood in plain tubes. | |
| | Plasma | Collect blood in EDTA tubes. | Plasma is preferred for low-abundance cytokines |
| | CSF | Collect 5–10 mL; discard the first five drops; use atraumatic needle and polypropylene tubes. | Avoid blood contamination; exclude samples with >500 RBCs/μL. |
| | Faecal | Collect 1–2 g in sterile containers | |
| | Saliva | Use passive drool method; ≥200 μL. | |
| **Timing of collection** | | | |
| | All | Prefer morning for blood collection; standardise timing when possible; always record collection time. | |
| **Sample handling** | | | |
| | Blood | Define intended use before processing. | Ensures correct tube and protocol selection. |
| | PBMCs | Isolate PBMCs using Ficoll-Paque, CPT, or Leucosep/ Lymphoprep tubes; document anticoagulant type and isolation method; process within 8 h at room temperature; do not exceed 24 h before processing. | CPT/Leucosep simplify isolation and reduces variability; manual Ficoll with EDTA yields higher PBMC counts; delayed processing impairs viability; cell number requirements depend on downstream assays. |
| | PMNCs | Use Percoll or sedimentation to isolate PMNCs; with limited blood sample availability, isolate PMNCs and PBMCs together via Ficoll-Leucosep, and combine post-isolation for immunophenotyping. | Percoll reduces neutrophil priming; neutrophils are highly sensitive to handling. |
| | Serum | Allow to clot ≥15 min, centrifuge within 60 min at 1000–2000×*g* for 10–15 min at 4 °C. | Delays and tube material affect measurements; serum cytokine levels may rise due to platelet activation |
| | Plasma | Centrifuge at 1000–2000×*g* for 10–15 min at 4 °C within 60 min of collection | |
| | CSF | Keep on ice; centrifuge at 500–1500×*g* for 10–15 min 4 °C within 60 min. | Use the cell pellet immediately for immunophenotyping to maximise cell number |
| | Faecal sample | Aliquot quickly | |
| **Storage** | | Avoid multiple freeze-thaw cycles | |
| | Blood | | |
| | PBMCs | Controlled-rate freezing with liquid nitrogen in 10% DMSO/ FCS; concentrate PBMCs >6 × $10^6$ cells/mL | Fresh PBMCs preserve maximal functionality; cryopreservation enables batch analysis for longitudinal/ multicenter studies; storage at –80 °C is detrimental to cell viability and genomic stability. |
| | Serum | Use low-binding polypropylene tubes, store at –80 °C; use small aliquots to avoid freeze-thaw. | |
| | Plasma | | |
| | CSF | Freeze pellet and supernatant at –80 °C. (*specific conditions are required for single-cell) | Pellet for RNA sequencing, for protein, extracellular vesicle, or cell-free RNA analysis |
| | Faecal sample | Aliquot and freeze at –80 °C within 2 h; temporary 4 °C overnight allowed. Avoid freeze-thaw cycles. | |
| | Saliva | Store at –80 °C in low-binding tubes in small aliquots (~200 μL). | |
| **Documentation** | All | Record collection time, processing time, deviations, and pre-analytical variables (e.g. medication, oral health). | Critical for reproducibility and adjustment of analyses |
| **Transport** | All | Maintain cold chain using dry ice or equivalent. | Prevents sample degradation during transfer. |

*CSF* cerebrospinal fluid, *PBMCs* peripheral blood mononuclear cells, *PMNCs* polymorphonuclear cells, *RBC* red blood cells.

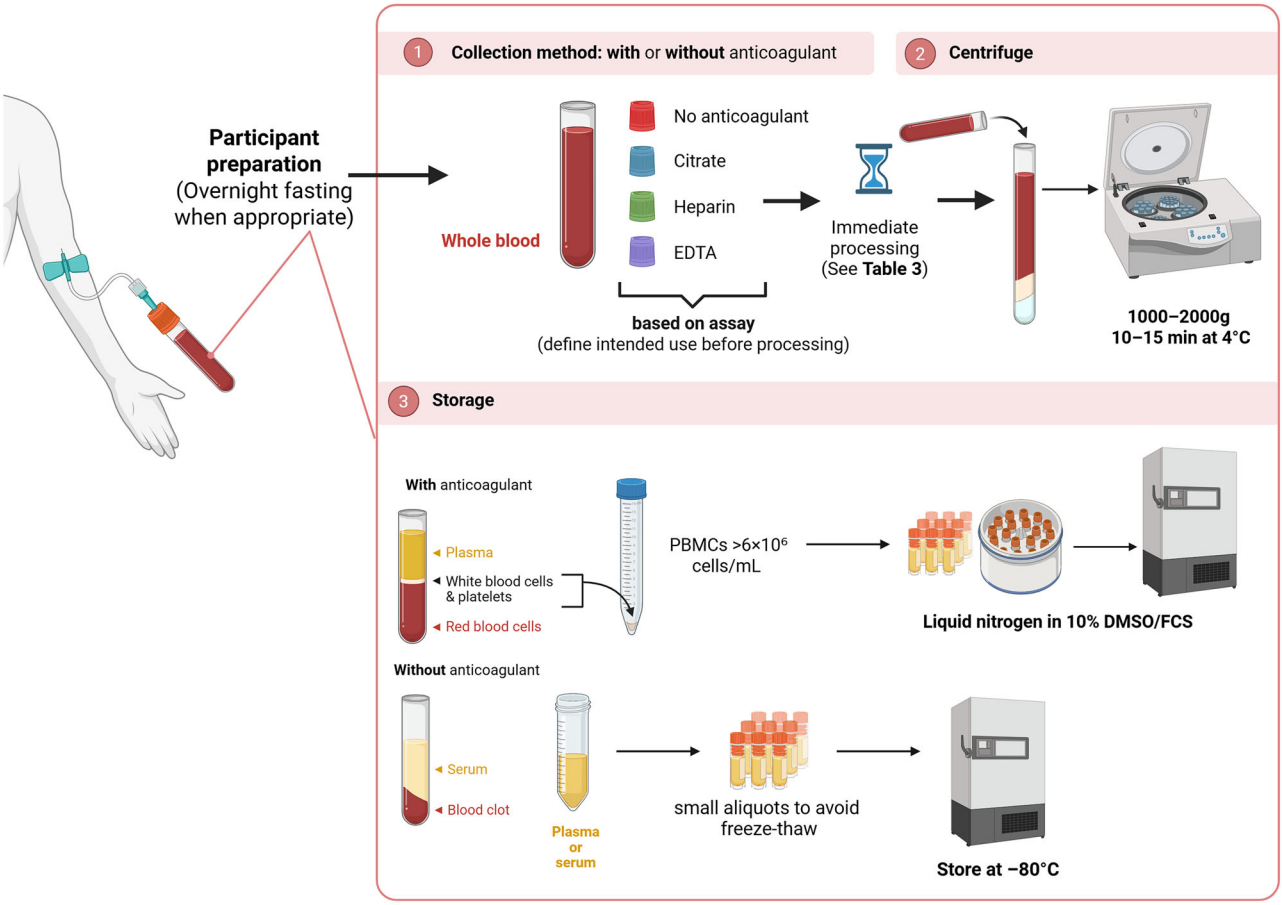


**Fig. 1** | Standardized workflow recommendations for blood sample handling.

and microbiota subpopulations, shedding light on how microbial changes may contribute to inflammatory processes and disease severity in PD[161]. However, stool analysis presents unique challenges due to the complex and heterogeneous nature of the human faecal matter and methodologies for assessing cytokine alterations remain poorly standardised[162].

Sample collection can be hampered by difficulties in collection (e.g. avoiding cross-contamination upon sample collection, or patient compliance with home sampling) as well as gut symptoms such as constipation in PD. For adequate sample collection, sterile non-reactive containers designed for the purpose should be used, ensuring that a representative sample of stool (typically 1–2 g) is collected, without being contaminated with urine or water. The faecal samples should be aliquoted to prevent degradation and unnecessary repeated freeze-thaw cycles. They should be cryopreserved (−80 °C) for long-term storage within a 2-h window after collection. As an alternative, they can be stored temporarily at 4 °C, but not longer than overnight[162].

### Saliva

There is increasing interest in using saliva to measure inflammatory biomarkers, as it is non-invasive, requires a lower biosafety classification compared to blood, and requires less specialised personnel to collect. Salivary inflammatory markers have scarcely been explored in PD[163]. The concentration of levels of inflammatory markers in saliva is relatively high, and some studies show concentrations higher than detected in blood[164]. There are some unique considerations when collecting saliva, such as oral health status (e.g. participants should be excluded if there is evidence of ulceration, dental abscess or recent dental work) and restrictions on behaviours prior to saliva collection (e.g. avoiding food and drink for 1 h prior to collection). Specific particularities of saliva collection, handling and processing are described elsewhere[164].

## Conclusions

Immune processes are strongly implicated in the pathogenesis of PD, though there is a lack of data from large-scale human studies on how immune markers map onto clinical subtypes and disease stages. Furthermore, there are considerable discrepancies in the results of clinical studies, and methodological heterogeneity in study design and sample collection may be limiting our progress and our ability to obtain reliable and robust data. This review aims to guide the scientific community in optimal design of observational studies to advance our understanding of the role of immunity in the development and progression of PD, with the ultimate goal of developing new immune-targeting disease-modifying therapies. This manuscript was developed by a panel of experts from the IMMUPARKNET consortium, integrating current evidence with consensus-based recommendations. As the field advances, future systematic reviews and formal evidence grading will be essential to further strengthen these guidelines as additional data becomes available.

### Data Sharing

Data sharing is not applicable to this article, as no datasets were generated or analysed during the current study

## Data availability

No datasets were generated or analysed during the current study.

Received: 18 November 2024; Accepted: 1 September 2025;
Published online: 20 October 2025

## References


1. Roodveldt, C. et al. The immune system in Parkinson's disease: what we know so far. *Brain* **147**, 3306–3324 (2024).
2. Gugu, M. et al. IMMUnity unveiled: a translational NETwork for tackling PARKinson's disease – IMMUPARKNET. *Open Res. Eur.* **4**, 119 (2025).
3. Postuma, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov. Disord.* **30**, 1591–1601 (2015).
4. Berg, D. et al. Movement disorder society criteria for clinically established early Parkinson's disease. *Mov. Disord.* **33**, 1643–1646 (2018).
5. Simuni, T. et al. A biological definition of neuronal α-synuclein disease: towards an integrated staging system for research. *Lancet Neurol.* **23**, 178–190 (2024).
6. Höglinger, G. U. et al. A biological classification of Parkinson's disease: the SynNeurGe research diagnostic criteria. *Lancet Neurol.* **23**, 191–204 (2024).
7. Zhang, H. et al. Plasma immune markers in an idiopathic REM sleep behavior disorder cohort. *Parkinsonism Relat. Disord.* **78**, 145–150 (2020).
8. Farmen, K. et al. Monocyte markers correlate with immune and neuronal brain changes in REM sleep behavior disorder. *Proc. Natl Acad. Sci. USA* **118**, e2020858118 (2021).
9. Heinzel, S. et al. Update of the MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* **34**, 1464–1470 (2019).
10. Thaler, A. et al. Mutations in GBA and LRRK2 are not associated with increased inflammatory markers. *J. Parkinsons Dis.* **11**, 1285–1296 (2021).
11. Dzamko, N., Rowe, D. B. & Halliday, G. M. Increased peripheral inflammation in asymptomatic leucine-rich repeat kinase 2 mutation carriers. *Mov. Disord.* **31**, 889–897 (2016).
12. Koros, C. et al. Peripheral Immune pattern in a genetic cohort of p.A53T alpha-synuclein carriers. *Parkinsonism Relat. Disord.* **135**, 107853 (2025).
13. Pawelec, G., Larbi, A. & Derhovanessian, E. Senescence of the human immune system. *J. Comp. Pathol.* **142**, S39–S44 (2010).
14. Klein, S. L. & Flanagan, K. L. Sex differences in immune responses. *Nat. Rev. Immunol.* **16**, 626–638 (2016).
15. Nissen, S. K. et al. Alterations in blood monocyte functions in Parkinson's disease. *Mov. Disord.* **34**, 1711–1721 (2019).
16. Carlisle, S. M. et al. Sex-based differences in the activation of peripheral blood monocytes in early Parkinson disease. *NPJ Parkinsons Dis.* **7**, 36 (2021).
17. Xiang, Y., Zhang, M., Jiang, D., Su, Q. & Shi, J. The role of inflammation in autoimmune disease: a therapeutic target. *Front. Immunol.* **14**, 1267091 (2023).
18. Liu, W. et al. Immune and inflammatory mechanisms and therapeutic targets of gout: an update. *Int. Immunopharmacol.* **121**, 110466 (2023).
19. Shin, E. C., Sung, P. & Park, S. H. Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat. Rev. Immunol.* **16**, 509–523 (2016).
20. Mac Giollabhui, N. et al. To exclude or not to exclude: considerations and recommendations for C-reactive protein values higher than 10 mg/L. *Brain Behav. Immun.* **87**, 898–900 (2020).
21. Carrión-Barberà, I. & Lood, C. Performance of the neutrophil-to-lymphocyte ratio as a prognostic tool for survival in solid cancers. *Front. Oncol.* **15**, 1616477 (2025).
22. Barrett, A. J. & Savani, B. N. Does chemotherapy modify the immune surveillance of hematological malignancies?. *Leukemia* **23**, 53–58 (2009).
23. Moreno, C. et al. Restoration of the immune function as a complementary strategy to treat chronic lymphocytic leukemia effectively. *J. Exp. Clin. Cancer Res.* **40**, 321 (2021).
24. Dąbrowska, A. M. & Słotwiński, R. The immune response to surgery and infection. *Cent. Eur. J. Immunol.* **39**, 532–537 (2014).
25. Osuka, A., Ogura, H., Ueyama, M., Shimazu, T. & Lederer, J. A. Immune response to traumatic injury: harmony and discordance of immune system homeostasis. *Acute Med. Surg.* **1**, 63–69 (2014).
26. Berbudi, A., Rahmadika, N., Tjahjadi, A. I. & Ruslami, R. Type 2 Diabetes and its Impact on the Immune System. *Curr. Diabetes Rev.* **16**, 442–449 (2020).
27. Wiseman, A. C. Immunosuppressive medications. *Clin. J. Am. Soc. Nephrol.* **11**, 332–343 (2016).
28. Butler, W. T. & Rossen, R. D. Effects of corticosteroids on immunity in man. I. Decreased serum IgG concentration caused by 3 or 5 days of high doses of methylprednisolone. *J. Clin. Invest.* **52**, 2629–2640 (1973).
29. Sastre, J. & Mosges, R. Local and systemic safety of intranasal corticosteroids. *J. Investig. Allergol. Clin. Immunol.* **22**, 1–12 (2012).
30. Pandya, D., Puttanna, A. & Balagopal, V. Systemic effects of inhaled corticosteroids: an overview. *Open Respir. Med. J.* **8**, 59–65 (2014).
31. Sakonlaya, D., Tapanadechopone, P., Poomkokruk, A. & Charoenvilaisiri, S. Do NSAIDs inhibit growth of precancerous cervical cells in vitro?. *J. Med. Assoc. Thai* **95**, S65–S73 (2012).
32. Morris, T. et al. Effects of low-dose aspirin on acute inflammatory responses in humans. *J. Immunol.* **183**, 2089–2096 (2009).
33. Pollard, A. J. & Bijker, E. M. A guide to vaccinology: from basic principles to new developments. *Nat. Rev. Immunol.* **21**, 83–100 (2021).
34. Yuan, X. et al. Peripheral inflammatory and immune landscape in multiple system atrophy: a cross-sectional study. *Mov. Disord.* **39**, 391–399 (2024).
35. Muñoz-Delgado, L. et al. Peripheral immune profile and neutrophil-to-lymphocyte ratio in progressive supranuclear palsy: case-control study and meta-analysis. *Eur. J. Neurol.* **31**, e16451 (2024).
36. Doroszkiewicz, J., Winkel, I. & Mroczko, B. Comparative analysis of neuroinflammatory pathways in Alzheimer's disease, Parkinson's disease, and multiple sclerosis: insights into similarities and distinctions. *Front. Neurosci.* **19**, 1579511 (2025).
37. Zhu, Y. et al. Association between inflammatory bowel diseases and Parkinson's disease: systematic review and meta-analysis. *Neural Regen. Res.* **17**, 344–353 (2022).
38. Witoelar, A. et al. Genome-wide pleiotropy between Parkinson disease and autoimmune diseases. *JAMA Neurol.* **74**, 780–792 (2017).
39. Magistrelli, L. et al. Relationship between circulating CD4+ T lymphocytes and cognitive impairment in patients with Parkinson's disease. *Brain Behav. Immun.* **89**, 668–674 (2020).
40. Marder, K., Tang, M. X., Cote, L., Stern, Y. & Mayeux, R. The frequency and associated risk factors for dementia in patients with Parkinson's disease. *Arch. Neurol.* **52**, 695–701 (1995).
41. Petty, R. et al. Improving recruitment and retention of people with Parkinson's disease to clinical studies: a scoping review. *J. Parkinson's Dis.* **15**, 6–18 (2025).
42. Teague, S. et al. Retention strategies in longitudinal cohort studies: a systematic review and meta-analysis. *BMC Med. Res. Methodol.* **18**, 151 (2018).
43. Weemering, D. N. et al. Trial participation in neurodegenerative diseases: barriers and facilitators: a systematic review and meta-analysis. *Neurology* **103**, e209503 (2024).
44. Jiang, S. et al. Cell ratio differences in peripheral blood between early- and late-onset Parkinson's disease: a case-control study. *Biomed. Res. Int.* **2019**, 2072635 (2019).

45. Freuchet, A., Pinçon, A., Sette, A. & Lindestam Arlehamn, C. S. Inflammation and heterogeneity in synucleinopathies. *Front. Immunol.* **15**, 1432342 (2024).
46. Lyn-Cook, B. D. et al. Increased expression of Toll-like receptors (TLRs) 7 and 9 and other cytokines in systemic lupus erythematosus (SLE) patients: ethnic differences and potential new targets for therapeutic drugs. *Mol. Immunol.* **61**, 38–43 (2014).
47. Martin, C. A. et al. Ethnic differences in cellular and humoral immune responses to SARS-CoV-2 vaccination in UK healthcare workers: a cross-sectional analysis. *EClinicalMedicine* **58**, 101926 (2023).
48. Mamais, A., Kaganovich, A. & Harvey, K. Convergence of signalling pathways in innate immune responses and genetic forms of Parkinson's disease. *Neurobiol. Dis.* **169**, 105721 (2022).
49. Wallings, R. L. & Tansey, M. G. LRRK2 regulation of immune-pathways and inflammatory disease. *Biochem. Soc. Trans.* **47**, 1581–1595 (2019).
50. Muñoz-Delgado, L. et al. Peripheral inflammatory immune response differs among sporadic and familial Parkinson's disease. *NPJ Parkinsons Dis.* **9**, 12 (2023).
51. Kozina, E. et al. Mutant LRRK2 mediates peripheral and central immune responses leading to neurodegeneration in vivo. *Brain* **141**, 1753–1769 (2018).
52. Collins, T. J. C. et al. The influence of metabolic disorders on adaptive immunity. *Cell Mol. Immunol.* **21**, 1109–1119 (2024).
53. Fernandez-Ruiz, I. rene Immune system and cardiovascular disease. *Nat. Rev. Cardiol.* **13**, 503 (2016).
54. Grossmann, V. et al. Profile of the immune and inflammatory response in individuals with prediabetes and type 2 diabetes. *Diabetes Care.* **38**, 1356–1364 (2015).
55. Kline, E. M. et al. Genetic and environmental factors in Parkinson's disease converge on immune function and inflammation. *Mov. Disord.* **36**, 25–36 (2021).
56. Kulcsarova, K., Bang, C., Berg, D. & Schaeffer, E. Pesticides and the microbiome-gut-brain axis: convergent pathways in the pathogenesis of Parkinson's disease. *J. Parkinsons Dis.* **13**, 1079–1106 (2023).
57. Smeyne, R. J., Noyce, A. J., Byrne, M., Savica, R. & Marras, C. Infection and risk of Parkinson's disease. *J. Parkinsons Dis.* **11**, 31–43 (2021).
58. Wu, D., Lewis, E. D., Pae, M. & Meydani, S. N. Nutritional modulation of immune function: analysis of evidence, mechanisms, and clinical relevance. *Front. Immunol.* **9**, 3160 (2019).
59. Nieman, D. C. & Wentz, L. M. The compelling link between physical activity and the body's defense system. *J. Sport Health Sci.* **8**, 201–217 (2019).
60. Besedovsky, L., Lange, T. & Born, J. Sleep and immune function. *Pflug. Arch.* **463**, 121–137 (2012).
61. Pinoli, M., Marino, F. & Cosentino, M. Dopaminergic regulation of innate ilmmunity: a review. *J. Neuroimmune Pharmacol.* **12**, 602–623 (2017).
62. Thomas Broome, S. et al. Dopamine: an immune transmitter. *Neural Regen. Res.* **15**, 2173–2185 (2020).
63. Kustrimovic, N., Rasini, E., Legnaro, M., Marino, F. & Cosentino, M. Expression of dopaminergic receptors on human CD4+ T lymphocytes: flow cytometric analysis of naive and memory subsets and relevance for the neuroimmunology of neurodegenerative disease. *J. Neuroimmune Pharmacol.* **9**, 302–312 (2014).
64. Tomlinson, C. L. et al. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov. Disord.* **25**, 2649–2653 (2010).
65. Lawton, M. et al. Blood biomarkers with Parkinson's disease clusters and prognosis: the Oxford discovery cohort. *Mov. Disord.* **35**, 279–287 (2020).
66. Reale, M. et al. Peripheral cytokines profile in Parkinson's disease. *Brain Behav. Immun.* **23**, 55–63 (2009).
67. Sun, C. et al. Peripheral humoral immune response is associated with the non-motor symptoms of Parkinson's disease. *Front Neurosci.* **13**, 1057 (2019).
68. Williams-Gray, C. H. et al. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). *Mov. Disord.* **31**, 995–1003 (2016).
69. Wijeyekoon, R. S. et al. Peripheral innate immune and bacterial signals relate to clinical heterogeneity in Parkinson's disease. *Brain Behav. Immun.* **87**, 473–488 (2020).
70. Scott, K. M. et al. B lymphocyte responses in Parkinson's disease and their possible significance in disease progression. *Brain Commun.* **5**, fcad060 (2023).
71. Scott, K. M., Kouli, A., Yeoh, S. L., Clatworthy, M. R. & Williams-Gray, C. H. A systematic review and meta-analysis of alpha synuclein auto-antibodies in Parkinson's disease. *Front. Neurol.* **9**, 815 (2018).
72. Lindestam Arlehamn, C. S. et al. α-Synuclein-specific T cell reactivity is associated with preclinical and early Parkinson's disease. *Nat. Commun.* **11**, 1875 (2020).
73. Bhidayasiri, R. & Martinez-Martin, P. Clinical assessments in Parkinson's disease: scales and monitoring. *Int. Rev. Neurobiol.* **132**, 129–182 (2017).
74. Hoehn, M. M. & Yahr, M. D. Parkinsonism: onset, progression and mortality. *Neurology* **17**, 427–442 (1967).
75. Goetz, C. G. et al. Movement Disorder Society UPDRS revision Task Force. Movement Disorder Society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov. Disord.* **23**, 2129–2170 (2008).
76. Kouli, A. et al. Neuroinflammation is linked to dementia risk in Parkinson's disease. *Brain* **147**, 923–935 (2024).
77. Litvan, I. et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. *Mov. Disord.* **27**, 349–356 (2012).
78. Emre, M. et al. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov. Disord.* **22**, 1689–1707 (2007).
79. Skorvanek, M. et al. Global scales for cognitive screening in Parkinson's disease: Critique and recommendations. *Mov. Disord.* **33**, 208–218 (2018).
80. Miller, A. H. & Raison, C. L. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* **16**, 22–34 (2016).
81. Houser, M. C. & Tansey, M. G. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *NPJ Parkinsons Dis*. **3**, 3 (2017).
82. Brown, G. C., Camacho, M. & Williams-Gray, C. H. The endotoxin hypothesis of Parkinson's disease. *Mov. Disord.* **38**, 1143–1155 (2023).
83. Camacho, M., Greenland, J. C. & Williams-Gray, C. H. The gastrointestinal dysfunction scale for Parkinson's disease. *Mov. Disord.* **36**, 2358–2366 (2021).
84. Doty, R. L. Olfactory dysfunction in Parkinson disease. *Nat. Rev. Neurol.* **8**, 329–339 (2012).
85. Brumm, M. C. et al. Updated percentiles for the University of Pennsylvania smell identification test in adults 50 years of age and older. *Neurology* **100**, e1691–e1701 (2023).
86. Rumeau, C., Nguyen, D. T. & Jankowski, R. How to assess olfactory performance with the Sniffin' Sticks test(®). *Eur. Ann. Otorhinolaryngol. Head. Neck Dis.* **133**, 203–206 (2016).
87. Chaudhuri, K. R. et al. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: results from an international pilot study. *Mov. Disord.* **22**, 1901–1911 (2007). Oct 15.
88. Lee, Y., Park, Y., Nam, H., Lee, J. W. & Yu, S. W. Translocator protein (TSPO): the new story of the old protein in neuroinflammation. *BMB Rep.* **53**, 20–27 (2020).

89. Kreisl, W. C. et al. PET imaging of neuroinflammation in neurological disorders. *Lancet Neurol.* **19**, 940–950 (2020).
90. Wijesinghe, S. S. et al. Post-mortem validation of in vivo TSPO PET as a microglial biomarker. *Brain* **148**, 1904–1910 (2025).
91. Zhang, P. F. & Gao, F. Neuroinflammation in Parkinson's disease: a meta-analysis of PET imaging studies. *J. Neurol.* **269**, 2304–2314 (2022).
92. Ouchi, Y. et al. Microglial activation and dopamine terminal loss in early Parkinson's disease. *Ann. Neurol.* **57**, 168–175 (2005).
93. Gerhard, A. et al. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol. Dis.* **21**, 404–412 (2006).
94. Yacoubian, T. A. et al. Brain and systemic inflammation in de novo Parkinson's disease. *Mov. Disord.* **38**, 743–754 (2023).
95. Stokholm, M. G. et al. Assessment of neuroinflammation in patients with idiopathic rapid-eye-movement sleep behaviour disorder: a case-control study. *Lancet Neurol.* **16**, 789–796 (2017).
96. Mullin, S. et al. Brain microglial activation increased in glucocerebrosidase (GBA) mutation carriers without Parkinson's disease. *Mov. Disord.* **36**, 774–779 (2021).
97. Li, M. et al. Dopamine, a co-regulatory component, bridges the central nervous system and the immune system. *Biomed. Pharmacother.* **145**, 112458 (2022).
98. Peyronneau, M. A. et al. [18F]DPA-714: effect of co-medications, age, sex, BMI and TSPO polymorphism on the human plasma input function. *Eur. J. Nucl. Med. Mol. Imaging* **50**, 3251–3264 (2023).
99. Jain, P. et al. Neuroinflammation PET Imaging: current opinion and future directions. *J. Nucl. Med.* **61**, 1107–1112 (2020).
100. Tan, L. Y. et al. Emergence of the brain-border immune niches and their contribution to the development of neurodegenerative diseases. *Front. Immunol.* **15**, 1380063 (2024).
101. Kanel, P., Carli, G., Vangel, R., Roytman, S. & Bohnen, N. I. Challenges and innovations in brain PET analysis of neurodegenerative disorders: a mini-review on partial volume effects, small brain region studies, and reference region selection. *Front. Neurosci.* **17**, 1293847 (2023).
102. Tansey, M. G. et al. Inflammation and immune dysfunction in Parkinson disease. *Nat. Rev. Immunol.* **22**, 657–673 (2022).
103. Schroder et al. Immune cell activation in the cerebrospinal fluid of patients with Parkinson's disease. *Front. Neurol.* **9**, 1081 (2018).
104. Pillny, C., Nitsch, L., Proske-Schmitz, S., Sharma, A. & Wüllner, U. Abnormal subpopulations of monocytes in the cerebrospinal fluid of patients with Parkinson's disease. *Parkinsonism Relat. Disord.* **84**, 144–145 (2021).
105. Qin, X. Y., Zhang, S. P., Cao, C., Loh, Y. P. & Cheng, Y. Aberrations in peripheral inflammatory cytokine levels in Parkinson disease: a systematic review and meta-analysis. *JAMA Neurol.* **73**, 1316–1324 (2016).
106. Qu, Y. et al. A systematic review and meta-analysis of inflammatory biomarkers in Parkinson's disease. *NPJ Parkinsons Dis.* **9**, 18 (2023).
107. Coogan, A. N. & Wyse, C. A. Neuroimmunology of the circadian clock. *Brain Res.* **1232**, 104–112 (2008).
108. Chen, Y. C., Wang, W. S., Lewis, S. J. G. & Wu, S. L. Fighting against the clock: circadian disruption and Parkinson's disease. *J. Mov. Disord.* **17**, 1–14 (2024).
109. Li, T. et al. Peripheral clock system abnormalities in patients with Parkinson's disease. *Front. Aging Neurosci.* **13**, 736026 (2021).
110. Scheiermann, C., Gibbs, J., Ince, L. & Loudon, A. Clocking in to immunity. *Nat. Rev. Immunol.* **18**, 423–437 (2018).
111. Wijeyekoon, R. S. et al. Cerebrospinal Fluid Cytokines and Neurodegeneration-Associated Proteins in Parkinson's Disease. *Mov. Disord.* **35**, 1062–1066 (2020).
112. Kofanova, O. et al. IL8 and EDEM3 gene expression ratio indicates peripheral blood mononuclear cell (PBMC) quality. *J. Immunol. Methods* **465**, 13–19 (2019).
113. Popko, K. et al. Influence of blood sample storage and different types of anticoagulants on results of NK cytotoxicity tests. *Cent. Eur. J. Immunol.* **48**, 267–273 (2023).
114. Efthymiou, A. et al. Isolation and freezing of human peripheral blood mononuclear cells from pregnant patients. *STAR Protoc.* **3**, 101204 (2022).
115. Zborowski, M. aciej, Moore, L. eeR., Melnik, K. ristie & Jing, Y. ing Worked examples of cell sample preparation and magnetic separation procedures. *Lab. Tech. Biochem. Mol. Biol.* **32**, 293–330 (2007).
116. Chen, H. et al. Functional comparison of PBMCs isolated by cell preparation tubes (CPT) vs. lymphoprep tubes. *BMC Immunol.* **21**, 15 (2020).
117. Betsou, F., Gaignaux, A., Ammerlaan, W., Norris, P. J. & Stone, M. Biospecimen science of blood for peripheral blood mononuclear cell (PBMC) functional applications. *Curr. Pathobiol. Rep.* **7**, 17–27 (2019).
118. Yi, P. C. et al. Impact of delayed PBMC processing on functional and genomic assays. *J. Immunol. Methods* **519**, 113514 (2023).
119. Olson, W. C. et al. Shipping blood to a central laboratory in multicenter clinical trials: effect of ambient temperature on specimen temperature, and effects of temperature on mononuclear cell yield, viability and immunologic function. *J. Transl. Med.* **9**, 26 (2011).
120. Thyagarajan, B. et al. Effect of delayed cell processing and cryopreservation on immunophenotyping in multicenter population studies. *J. Immunol. Methods* **463**, 61–70 (2018).
121. Higdon, L. et al. Impact on in-depth immunophenotyping of delay to peripheral blood processing. *Clin. Exp. Immunol.* **217**, 119–132 (2024).
122. Martire, S. et al. The impact of pre-freezing storage time and temperature on gene expression of blood collected in EDTA tubes. *Mol. Biol. Rep.* **49**, 4709–4718 (2022).
123. Costantini, A. et al. Effects of cryopreservation on lymphocyte immunophenotype and function. *J. Immunol. Methods* **278**, 145–155 (2003).
124. Jeurink, P. V., Vissers, Y. M., Rappard, B. & Savelkoul, H. F. T cell responses in fresh and cryopreserved peripheral blood mononuclear cells: kinetics of cell viability, cellular subsets, proliferation, and cytokine production. *Cryobiology* **57**, 91–103 (2008).
125. Usero, L. et al. Feasibility of using monocyte-derived dendritic cells obtained from cryopreserved cells for DC-based vaccines. *J. Immunol. Methods* **498**, 113133 (2021).
126. Reimann, K. A., Chernoff, M., Wilkening, C. L., Nickerson, C. E. & Landay, A. L. Preservation of lymphocyte immunophenotype and proliferative responses in cryopreserved peripheral blood mononuclear cells from human immunodeficiency virus type 1-infected donors: implications for multicenter clinical trials. The ACTG immunology advanced technology laboratories. *Clin. Diagn. Lab Immunol.* **7**, 352–359 (2000).
127. Draxler, D. F., Madondo, M. T., Hanafi, G., Plebanski, M. & Medcalf, R. L. A flowcytometric analysis to efficiently quantify multiple innate immune cells and T Cell subsets in human blood. *Cytom. A.* **91**, 336–350 (2017).
128. Marino, M. et al. Impact of 12-month cryopreservation on endogenous DNA damage in whole blood and isolated mononuclear cells evaluated by the comet assay. *Sci. Rep.* **11**, 363 (2021).
129. Ticha, O., Moos, L. & Bekeredjian-Ding, I. Effects of long-term cryopreservation of PBMC on recovery of B cell subpopulations. *J. Immunol. Methods* **495**, 113081 (2021).
130. Li, B. et al. Comprehensive evaluation of the effects of long-term cryopreservation on peripheral blood mononuclear cells using flow cytometry. *BMC Immunol.* **23**, 30 (2022).
131. Barcelo, H., Faul, J., Crimmins, E. & Thyagarajan, B. A Practical cryopreservation and staining protocol for immunophenotyping in population studies. *Curr. Protoc. Cytom.* **84**, e35 (2018).

132. Disis, M. L. et al. Maximizing the retention of antigen specific lymphocyte function after cryopreservation. *J. Immunol. Methods* **308**, 13–18 (2006).
133. Bogoslovsky, T. et al. Cryopreservation and enumeration of human endothelial progenitor and endothelial cells for clinical trials. *J. Blood Disord. Transfus.* **4**, 158 (2013).
134. Yang, J. et al. The effects of storage temperature on PBMC gene expression. *BMC Immunol.* **17**, 6 (2016).
135. Fowke, K. R., Behnke, J., Hanson, C., Shea, K. & Cosentino, L. M. Apoptosis: a method for evaluating the cryopreservation of whole blood and peripheral blood mononuclear cells. *J. Immunol. Methods* **244**, 139–144 (2000).
136. Hope, C. M. et al. Optimization of blood handling and peripheral blood mononuclear cell cryopreservation of low cell number samples. *Int. J. Mol. Sci.* **22**, 9129 (2021).
137. Cossarizza, A. et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition). *Eur. J. Immunol.* **51**, 2708–3145 (2021).
138. Chan, L. L. et al. Accurate measurement of peripheral blood mononuclear cell concentration using image cytometry to eliminate RBC-induced counting error. *J. Immunol. Methods* **388**, 25–32 (2013).
139. Bagamery, K., Kvell, K., Landau, R. & Graham, J. Flow cytometric analysis of CD41-labeled platelets isolated by the rapid, one-step OptiPrep method from human blood. *Cytom. A.* **65**, 84–87 (2005).
140. McFarland, D. C., Zhang, C., Thomas, H. C. & Ratliff, T. L. Confounding effects of platelets on flow cytometric analysis and cell-sorting experiments using blood-derived cells. *Cytom. A.* **69**, 86–94 (2006).
141. Spurgeon, B. E. J., Linden, M. D., Michelson, A. D. & Frelinger, A. L. 3rd. Immunophenotypic analysis of platelets by flow cytometry. *Curr. Protoc.* **1**, e178 (2021).
142. Sroka, J., Kordecka, A., Włosiak, P., Madeja, Z. & Korohoda, W. Separation methods for isolation of human polymorphonuclear leukocytes affect their motile activity. *Eur. J. Cell Biol.* **88**, 531–539 (2009).
143. Kuhns, D. B., Priel, D. A. L., Chu, J. & Zarember, K. A. Isolation and functional analysis of human neutrophils. *Curr ProtocImmunol* **111**, 7.23.1–7.23.16 (2015).
144. Kuang, Y., Parthasarathy, U. & Martinelli, R. Protocol for density gradient neutrophil isolation and flow cytometry-based characterization from human peripheral blood. *STAR Protoc.* **4**, 102497 (2023).
145. Alvieri, F. et al. Methods of granulocyte isolation from human blood and labeling with multimodal superparamagnetic iron oxide nanoparticles. *Molecules* **25**, 765 (2020).
146. Mosca, T. & Forte, W. C. Comparative efficiency and impact on the activity of blood neutrophils isolated by percoll, ficoll and spontaneous sedimentation methods. *Immunol. Invest.* **45**, 29–37 (2016).
147. Cui, C., Schoenfelt, K. Q., Becker, K. M. & Becker, L. Isolation of polymorphonuclear neutrophils and monocytes from a single sample of human peripheral blood. *STAR Protoc.* **2**, 100845 (2021).
148. Rosenberg-Hasson, Y., Hansmann, L., Liedtke, M., Herschmann, I. & Maecker, H. T. Effects of serum and plasma matrices on multiplex immunoassays. *Immunol. Res.* **58**, 224–233 (2014).
149. Burska, A., Boissinot, M. & Ponchel, F. Cytokines as biomarkers in rheumatoid arthritis. *Mediators Inflamm.* **2014**, 545493 (2014).
150. Liu, C. et al. Cytokines: from clinical significance to quantification. *Adv. Sci.* **8**, e2004433 (2021).
151. Friebe, A. & Volk, H. D. Stability of tumor necrosis factor alpha, interleukin 6, and interleukin 8 in blood samples of patients with systemic immune activation. *Arch. Pathol. Lab Med.* **132**, 1802–1806 (2008).
152. Aziz, N. et al. Stability of cytokines, chemokines and soluble activation markers in unprocessed blood stored under different conditions. *Cytokine* **84**, 17–24 (2016).
153. Gottfried-Blackmore, A. et al. Effects of processing conditions on stability of immune analytes in human blood. *Sci. Rep.* **10**, 17328 (2020).
154. Saugstad, J. A. et al. Analysis of extracellular RNA in cerebrospinal fluid. *J. Extracell. Vesicles.* **6**, 1317577 (2017).
155. Fiszer, U., Mix, E., Fredrikson, S., Kostulas, V. & Link, H. Parkinson's disease and immunological abnormalities: increase of HLA-DR expression on monocytes in cerebrospinal fluid and of CD45RO+ T cells in peripheral blood. *Acta Neurol. Scand.* **90**, 160–166 (1994).
156. Lepennetier, G. et al. Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases. *J. Neuroinflammation*. **16**, 219 (2019).
157. Faizan, M. et al. Cerebrospinal fluid protein biomarkers in Parkinson's disease. *Clin. Chim. Acta* **556**, 117848 (2024).
158. Cantoni, C. et al. A single-cell compendium of human cerebrospinal fluid identifies disease-associated immune cell populations. *J. Clin. Invest.* **135**, e177793 (2025).
159. Kodali, M. C. et al. Cryopreservation of cerebrospinal fluid cells preserves the transcriptional landscape for single-cell analysis. *J. Neuroinflammation* **21**, 71 (2024).
160. Lin, C. H. et al. Altered gut microbiota and inflammatory cytokine responses in patients with Parkinson's disease. *J. Neuroinflammation.* **16**, 129 (2019).
161. Houser, M. C. et al. Stool immune profiles evince gastrointestinal inflammation in Parkinson's disease. *Mov. Disord.* **33**, 793–804 (2018).
162. Boertien, J. M., Pereira, P. A. B., Aho, V. T. E. & Scheperjans, F. Increasing comparability and utility of gut microbiome studies in Parkinson's disease: a systematic review. *J. Parkinsons Dis.* **9**, S297–S312 (2019).
163. De Bartolo, M. I. et al. A systematic review of salivary biomarkers in Parkinson's disease. *Neural Regen. Res.* **19**, 2613–2625 (2024).
164. Szabo, Z. & Slavish, D. C. Measuring salivary markers of inflammation in health research: a review of methodological considerations and best practices. *Psychoneuroendocrinology* **124**, 105069 (2021).


## Acknowledgements

This publication is based upon work from COST Action CA21117, The role of IMMUnity in tackling PARKinson's disease through a Translational NETwork [IMMUPARKNET], supported by COST (European Cooperation in Science and Technology) (www.cost.eu). Laura Muñoz-Delgado is financially supported by the "Juan Rodés" programme (JR23/00016) from the Instituto de Salud Carlos III and receives funding support from the Consejería de Salud y Consumo de la Junta de Andalucía (NEU-0005-2022). Caroline Williams-Grey receives financial support from the NIHR Cambridge Biomedical Research Centre (NIHR203312) and the Cambridge Centre for Parkinson-Plus, and grant funding from the UKRI Medical Research Council (MR/W029235/1), Cure Parkinson's, Parkinson's UK, and the Rosetrees Trust. Gaetan Garraux is supported by 'Fonds Rahier', University of Liège and Fonds de la Recherche Scientifique (F.R.S.-FNRS) de Belgique. Gaëtan Garraux has also received support for travel or honorarium from AbbVie, Eurogenerics and Zambon. Diego Clemente has received funding support from Instituto de Salud Carlos III (PI18/00357, PI21/00302, PI24/00447, co-funded by the European Union, CIBERNED CB22/05/00016, "INVESTIGO" programme in the frame of the Plan para la Recuperación, Transformación y Resiliencia (European Union Next Generation funds), and EMD Serono R&D Agreement). Ines Figueira is supported by the the ERC—Grant No. 804229; through PeX—2022.02127.PTDC (PERCEPT) and through the

R&D unit iNOVA4Health (LISBOA-01-0145-FEDER-007344; UIDB/04462/2020), and LS4FUTURE Associated Laboratory (LA/P/0087/2020).Pablo Mir has received financial support from the Spanish Ministry of Science and Innovation (RTC2019-007150-1), the Instituto de Salud Carlos III-Fondo Europeo de Desarrollo Regional (ISCIII-FEDER) (PI16/01575, PI18/01898, PI19/01576, PI20/00613, PI21/01875, PI22/01704 and PI23/00512), the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía (CVI-02526, CTS-7685), the Consejería de Salud y Bienestar Social de la Junta de Andalucía (PI-0471-2013, PE-0210-2018, PI-0459-2018 and PE-0186-2019), the Consejería de Transformación Económica, Industria, Conocimiento y Universidades de la Junta de Andalucía (PY20_00896). Pablo Mir has also received support for attending meetings and/or travel or honorarium for lecturing from Abbott, Allergan, AbbVie, Bial, Britannia, Italfarmaco, Merz, UCB, Teva and Zambon. Cristoforo Comi is supported by the AGING PROJECT—Department of Excellence—Università del Piemonte Orientale. Cristoforo Comi has received travel grants for meeting participation from Ecupharma, Bial and Novartis.

## Author contributions

Concept and design: L.M.-D, C.H.W.-G, P.M. and C.C.; original draft preparation: L.M.-D, C.H.W.-G., G.G., D.C., O.O.Ç., J.C.G., I.F., I.M.-D, E.P., A.d.F., C.T. and S.E.; review and editing: L.M.-D., C.H.W.-G., G.G., D.C., I.F., E.P., A.d.F., S.P., P.M. and C.C. All authors read and approved the final submission.

## Competing interests

The authors declare no competing interests.

## Additional information

**Correspondence** and requests for materials should be addressed to Laura Muñoz-Delgado.