Antibody responses and risk factors associated with impaired immunological outcomes following two doses of BNT162b2 COVID-19 vaccination in patients with chronic pulmonary diseases

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ABSTRACT

Introduction Responses to COVID-19 vaccination in patients with chronic pulmonary diseases are poorly characterised. We aimed to describe humoral responses following two doses of BNT162b2 mRNA COVID-19 vaccine and identify risk factors for impaired responses.

Methods Prospective cohort study including adults with chronic pulmonary diseases and healthcare personnel as controls (1:1). Blood was sampled at inclusion, 3 weeks, 2 and 6 months after first vaccination. We reported antibody concentrations as geometric means with 95% CI of receptor binding domain (RBD)-IgG and neutralising antibody index of inhibition of ACE-2/RBD interaction (%). A low responder was defined as neutralising index in the lowest quartile (primary outcome) or RBD-IgG <225 AU/ml plus neutralising index <25% (secondary outcome), measured at 2 months. We tested associations using Poisson regression.

Results We included 593 patients and 593 controls, 75% of all had neutralising index ≥97% at 2 months. For the primary outcome, 34.7% of patients (n=157/453) and 12.9% of controls (n=46/359) were low responders (p<0.001). For the secondary outcome, 34.7% of patients (n=157/453) and 12.9% of controls (n=46/359) were low responders (p<0.001). Risk factors associated with low responder included increasing age (per decade, adjusted risk ratio (aRR) 1.17, 95%CI 1.03 to 1.32, Charlson Comorbidity Index (per point) (aRR 1.15, 95%CI 1.05 to 1.26), use of prednisolone (aRR 2.08, 95%CI 1.55 to 2.77) and other immunosuppressives (aRR 2.21, 95%CI 1.65 to 2.97).

Discussion Patients with chronic pulmonary diseases established functional humoral responses to vaccination, however lower than controls. Age, comorbidities and immunosuppression were associated with poor immunological responses.

WHAT IS ALREADY KNOWN ON THIS TOPIC
⇒ Today, close to 545 million people live with chronic pulmonary diseases, making such diseases some of the leading causes of morbidity and mortality worldwide. Patients with chronic pulmonary diseases are among the most susceptible individuals to developing severe and critical COVID-19. Until now, responses to COVID-19 vaccination in patients with chronic pulmonary diseases are poorly characterised.

WHAT THIS STUDY ADDS
⇒ This is the first study that explores humoral responses following two doses of mRNA BNT162b2 COVID-19 vaccination for up to 6 months.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY
⇒ Most patients with chronic pulmonary diseases responded adequately to vaccine, however, humoral responses were lower compared with controls. Furthermore, we found that age, comorbidities and use of systemic corticosteroids and other immunosuppressants, were all independently associated with having a lower response to vaccination.

INTRODUCTION
Patients with chronic pulmonary diseases, including chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), severe asthma and bronchiectasis, are at increased risk of severe and critical COVID-19.14 In addition, several retrospective cohort studies and case
series have reported an increased risk of hospitalisation, intensive care unit admission and mortality related to severe COVID-19 in patients with chronic pulmonary diseases,\(^{4,5}\) with risk estimates differing between disease groups. Thus, protective measures, including vaccination of individuals with chronic pulmonary diseases, are crucial in preventing morbidity and mortality related to SARS-CoV-2 infection.

High vaccine effects after two doses of mRNA COVID-19 vaccine have been reported from both phase III randomised placebo controlled studies and large population observational studies.\(^{6-11}\) Over time, a gradual decline in vaccine efficacy has been described, raising concerns about the sustained long-term protection conferred by vaccination and the subsequent introduction of an additional third or fourth vaccine dose in several countries.\(^{12-15}\) Published studies indicate that vaccine efficacy estimates reported from non-immunocompromised patients with chronic diseases are similar between risk groups.\(^{12,16,17}\) However, the characteristics and dynamics of humoral responses after mRNA COVID-19 vaccination in patients with chronic pulmonary diseases remain to be described.

We aimed to describe humoral responses following two doses of BNT162b2 mRNA COVID-19 vaccination and identify risk factors for impaired immunological responses in patients with chronic pulmonary diseases since this is a large group of patients with increased risk for severe COVID-19.

**PATIENTS AND METHODS**

**Setting and study design**

We conducted a prospective cohort study. Patients attending an outpatient clinic at the Department of Pulmonary Medicine at Herlev, Gentofte or North Zealand University Hospitals in Copenhagen, Capital Region, Denmark, from 15 January 2021 to 31 May 2021, were invited to participate in the study. The BNT162b2 mRNA COVID-19 vaccine (Comirnaty, Pfizer-BioNTech) was administered free of charge and used as one of the COVID-19 vaccines recommended by the Danish Health Authority in the vaccination programme.\(^{16}\) Participation in the study did not alter the vaccination timing or schedule.

The design and report of the study were done following the Strengthening the Reporting of Observational Studies in Epidemiology statement.\(^{18}\)

**Participants**

**Inclusion criteria**

Patients aged 18 years and older, attending one of the outpatient’s clinics at one of the study sites due to any of the following diagnoses of chronic pulmonary diseases (all requiring respiratory medicine specialist treatment): COPD, \(\alpha\)-1 antitrypsin deficiency, ILD (including idiopathic pulmonary fibrosis), sarcoidosis, severe asthma, bronchiectasis or sleep apnoea. Healthcare personnel aged 18 years and older, who have received the BNT162b2 mRNA COVID-19 vaccine were included as the control population, as previously described.\(^{20}\) Approximately 89.7% of the population has Danish origin.\(^{21}\)

**Exclusion criteria**

SARS-CoV-2 laboratory-confirmed infection determined by the presence of antibodies against the nucleocapsid protein (N-protein) before vaccination or during follow-up. To compare antibody responses between patients and controls, we matched by sex and the nearest age at the time of first vaccination (1:1).

**Exposures, variables and outcomes**

**Exposure**

BNT162b2 mRNA COVID-19 vaccine.

**Variables**

Age, sex, diagnosis of underlying chronic pulmonary disease, comorbidities (estimated by Charlson Comorbidity Index (CCI)), lung function expressed as the forced expired volume in the first second (FEV1), body mass index (BMI), immunosuppression (eg, use of oral steroids, another immunosuppressive drug (Anatomical Therapeutic Chemical codes L04), inhaled corticosteroids (ICS) and antifibrotic drugs. In addition, we categorised patients with the following diagnoses: (1) COPD, \(\alpha\)-1 antitrypsin deficiency, asthma, bronchiectasis and sleep apnoea as obstructive lung diseases (OLD); (2) patients with diffuse parenchymal pulmonary disease and sarcoidosis as ILD.

**Follow-up**

Baseline (from inclusion and to up to 13 days after the first dose), at 3 weeks (from 14 days and up to 33 days after the first dose and before administration of a second dose), at 2 months (between 34 days and up to 90 days after the first dose and only after administration of a second dose); and at 6 months (from 91 days and up to 273 days after the first dose). These time points correspond to the median time of the obtained samples.

**Outcomes**

Defined as a responder or low responder after two BNT162b2 mRNA COVID-19 vaccine doses. In the absence of an internationally validated cut-off value for a serological correlate of protection, we defined arbitrary outcomes based on current data suggesting that postimmunisation antibody levels and neutralising activity can be used as a valid measure to estimate short-term protection.\(^{22-24}\) Therefore, we defined the primary outcome as the antibody neutralising activity alone, expressed as the percentage (%) of inhibition of ACE-2 host receptor and the spike-glycoprotein receptor-binding domain (RBD) of SARS-CoV-2 interaction. For the primary outcome, a low responder was defined as an individual having a neutralising antibody index in the lowest quartile of the study population measured at least 2 weeks after the second dose of BNT162b2 mRNA COVID-19 vaccine.
(categorised as ‘2 months sample’). As a secondary outcome, we defined low responder as a combined outcome based on (1) the detection of RBD IgG antibodies expressed as arbitrary unit per mL (AU/mL)<225 AU/mL, concomitantly with (2) the detection of neutralising antibodies index <25% measured at least 2 weeks after the second dose of BNT162b2 mRNA COVID-19 vaccine (categorised as ‘2 months sample’). Laboratory analyses were performed as previously described.  

Data sources and statistical methods
Baseline clinical information was retrieved from patients’ medical files and for healthcare personnel from questionnaires fulfilled by study subjects at study entry. We matched patients and controls by the nearest age at the time of first vaccination in a 1:1 ratio using nearest neighbour matching with the Optmatch package in R.27 28 Continuous data were reported as medians with IQR, and differences were assessed by Mann-Whitney U test or t-test, as most appropriate. Categorical data were reported as frequency counts and percentages, and differences were evaluated using the $\chi^2$ test or Fisher’s exact test, as most appropriate. Missing data were handled by using complete-case analysis.

Sample size
We estimated that we needed to include 500 patients with chronic pulmonary diseases to detect a clinically significant risk increase for being a low responder (primary outcome) of at least an OR of 1.4 or more, using a significant risk increase for being a low responder (primary outcome based on (1) the detection of RBD IgG or mean neutralising antibodies index, the GMC of anti-RBD IgG or mean neutralising index with 95% CI at each visit was plotted for each sample.

For the immunological assays, we reported RBD IgG antibody levels as geometric mean concentrations (GMC) with 95% CI, and neutralising antibodies were reported as the neutralising index (%) of inhibition of ACE-2 and RBD interaction. We calculated the proportion of responders for the primary and secondary outcomes and compared it between patients and controls. To visualise the observed antibody concentration and neutralising index, the GMC of anti-RBD IgG or mean neutralising index with 95% CI at each visit was plotted for each sample. Poisson regression with robust SEs was used to test associations between humoral response and independent variables. In the multivariable models, we adjusted for age (per decade increase), sex, CCI (per point increase), immunosuppression (none, oral steroids, other drugs), FEV1 (increase per litre), use of ICS versus none, and use of antifibrotic drugs versus none. P values <0.05 were considered significant. Statistical analyses were performed using R V.3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

Patient and public involvement
Patients were not involved in developing the research question and outcome measures, design, recruitment and conduction of the study. The laboratory analysis results have been made available to study participants through electronic patient records.

RESULTS
Baseline characteristics
We included 626 patients diagnosed with chronic pulmonary disease who received a BNT162b2 mRNA COVID-19 vaccine, 33 patients were excluded since they only received one dose of vaccine, 593 were included in the further analysis. Baseline characteristics of patients and controls are shown in table 1. Compared with controls, patients with chronic pulmonary diseases were older, were more often males and had higher BMIs. The interval between the first and second vaccine dose was shorter for patients than for controls. Among patients, 67% (n=398/593) had OLD, 30% (n=183/593) had ILD, 8% (n=50/593) had both, 27% (n=160/593) had moderate to high levels of comorbidities (CCI ≥2). At the time of vaccination, 13% (n=76/593) were on systemic oral steroids (excluding those given during acute exacerbation), 11% (n=64/593) were on other immunosuppressive drugs, 7.4% (n=44/593) on anti-fibrotic agents, 29% (n=170/593) on ICS, 5% (n=30/593) were active smokers, 51% (n=305/593) were previous smokers.

Primary and secondary humoral outcomes in patients and controls
Humoral responses for the primary and secondary outcomes at different sampling time points during follow-up are shown in table 2. After two doses of the BNT162b2 mRNA COVID-19 vaccine, the three upper

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of patients with chronic pulmonary diseases and controls included in the study</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td>N</td>
<td>593</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>68 (58–74)</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>283 (47.7)</td>
</tr>
<tr>
<td>Median time between first and second vaccine dose, days, (IQR)</td>
<td>23 (22–25)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>27.2 (6.4)</td>
</tr>
<tr>
<td>BMI, body mass index.</td>
<td></td>
</tr>
</tbody>
</table>
Antibody profiles in patients and controls

Changes in the antibody concentrations and neutralising antibody index with 95% CI of the mean at different sampling time points during follow-up are shown in figures 1 and 2. Antibody concentrations in patients and controls increased significantly from baseline to 3 weeks after the first dose and at 2 months sample (figure 1). There was a decline in measured antibody concentrations from 2 to 6 months after the first vaccine dose in both patients and controls (figure 2).

Risk factors associated with a lower response to vaccination in patients with chronic pulmonary diseases

Risk ratio (RR) from the univariate and multivariate analysis, including factors of clinical importance associated with being a low responder in patients with chronic pulmonary disease at 2 months of follow-up are shown in table 3. Increasing age per 10 years (crude RR (cRR) 1.19, 95% CI 1.05 to 1.35; adjusted RR (aRR) 1.17, 95% CI 1.03 to 1.32), an increase in CCI (cRR 1.20, 95% CI 1.10 to 1.31; aRR 1.15, 95% CI 1.05 to 1.26), use of prednisolone (cRR 2.12, 95% CI 1.59 to 2.81; aRR 2.08, 95% CI 1.55 to 2.77) and use of other immunosuppressive drugs (cRR 2.04, 95% CI 1.52 to 2.72; aRR 2.21, 95% CI 1.65 to 2.97) were significantly associated with an increased risk for being a low responder.

DISCUSSION

In this prospective cohort study, including patients with chronic pulmonary diseases who were vaccinated with two doses of the BNT162b2 mRNA COVID-19 vaccine, we found that most patients could establish functional humoral responses to vaccination characterised by high antibody titres and high levels of neutralising antibodies. However, humoral responses were lower at 2 and 6 months in patients with chronic pulmonary diseases than those observed in controls, and varied at different quartiles of the total study population had a detectable neutralising index of 97%. For the primary outcome, 34.7% patients (n=157/453) and 12.9% of controls (n=46/359) were low responders (p<0.0001). For the secondary outcome, 8.6% patients (n=39/453) and 1.4% of controls (n=5/359) were low responders (p<0.0001). At 2 and 6 months of follow-up, a significantly higher proportion of patients with chronic pulmonary diseases were low responders following two doses of the BNT162b2 mRNA COVID-19 vaccine than controls for both the primary and secondary outcomes (table 2). For the secondary outcome, the differences in humoral responses were also significant after the first dose of the vaccine (table 2).
sampling points. We also identified risk factors of clinical importance for an impaired response to vaccination in patients with chronic pulmonary diseases, including increasing age, having more underlying comorbidities and using oral steroids or other immunosuppressive drugs. However, these results must be interpreted with caution in the absence of correlates of protection against severe outcomes when we are report our results.

In patients with chronic pulmonary diseases and controls, we observed high antibody titres and neutralising antibody index when the peak of immunity after the mRNA BNT162b2 vaccine would be expected. An explanation for the use of two different immunological outcomes for assessing the results of humoral responses in our study is that at the time we designed the study, conducted and reported the analysis, there was no...
Vaccine effectiveness might also be reduced in humoral responses elicited by COVID-19 patients with chronic pulmonary diseases.

Conducting epidemiological studies assessing vaccine efficacy against infection, hospitalisation and death in patients with chronic pulmonary disease is unknown. These results should be validated by levels of protection, the specific link to clinical protection.

Patients with chronic pulmonary disease may have lower immunity after vaccination, which is highly relevant for international consensus on the definition of correlates of protection after COVID-19 vaccination, either on the seropositivity threshold or the level of neutralising antibodies. Therefore, we considered it reasonable to define our primary outcome based on the measurement of neutralising index alone as an indicator of functional mediation of protection. We determined this threshold as the neutralising antibody level measured in the lower quartile of the study population. This showed to be a conservative assumption since several groups have reported a neutralising index >50% or median neutralising antibody titres (NT50) as thresholds. Thus, other thresholds for protection will lead to different estimates of humoral responses.

A recently published analysis of the immune correlate of mRNA-1273 COVID-19 vaccine from the efficacy trial indicated that vaccine efficacy increases with higher antibody titres and is highly mediated by neutralising antibodies. Based on this consideration, we defined a secondary outcome as a composite outcome that combined antibody titres and neutralising antibodies.

In both cases, by using any of the defined immunological outcomes, we found that humoral responses were consistently lower in patients with chronic pulmonary diseases compared with controls at 2 and 6 months after the first dose. Even though our results indicate that patients with chronic pulmonary diseases may have lower levels of protection, the specific link to clinical protection is unknown. These results should be validated by conducting epidemiological studies assessing vaccine efficacy against infection, hospitalisation and death in patients with chronic pulmonary diseases.

Several observational studies have described the decline in humoral responses elicited by COVID-19 vaccines over time, with the subsequent risk of breakthrough infections. Vaccine effectiveness might also be reduced against current and forthcoming SARS-CoV-2 variants of concern. Our results support that waning humoral immunity also occurs in individuals with chronic pulmonary diseases, similar to what we observed in the control population. While there have been conflicting reports on the risk of SARS-CoV-2 infection in patients with chronic pulmonary diseases, especially in asthma patients, their increased risk of severe outcomes after infection is well established. Several industrialised countries have introduced additional third and fourth doses to their COVID-19 vaccination schedules in light of the waning immunity after vaccination, which is highly relevant for patients with chronic pulmonary diseases.

The identified risk factors for being a low responder have not been extensively characterised for other groups than severely immunocompromised patients. Iatrogenic immunosuppression, including corticosteroids, has markedly reduced humoral and cellular immunogenicity of mRNA COVID-19 vaccines compared with healthy controls. We did not find evidence to support that anti-fibrotic drugs of inhalation corticosteroids were associated with poorer immunological outcomes after vaccination. Interestingly, similar findings have been reported from patients with autoimmune diseases, especially in asthma patients, with lung involvement, which are more likely to receive immunosuppression. Current guidelines for vaccination of immunocompromised patients recommend that vaccines should be administered before planned immunosuppression if feasible. Such considerations should be based on the risk of severe COVID-19 infection with and without immunological responses and the risk of pausing immunosuppression.

Some potential limitations to our study deserve careful consideration. First, we do not have information on the cause of the lost to follow-up of some patients. Most severely ill patients have been prioritised for COVID-19 vaccinations. Therefore, we considered it reasonable to define our primary outcome based on the measurement of neutralising index alone as an indicator of functional mediation of protection. We determined this threshold as the neutralising antibody level measured in the lower quartile of the study population. This showed to be a conservative assumption since several groups have reported a neutralising index >50% or median neutralising antibody titres (NT50) as thresholds. Thus, other thresholds for protection will lead to different estimates of humoral responses.

Table 3

<table>
<thead>
<tr>
<th>Age (per 10 years increase)</th>
<th>Crude risk rate (95% CI)</th>
<th>P value</th>
<th>Adjusted risk rate (95% CI)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>1.19 (1.05 to 1.35)</td>
<td>0.007</td>
<td>1.17 (1.03 to 1.32)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sex (male)</td>
<td>1.20 (1.10 to 1.31)</td>
<td>&lt;0.001</td>
<td>1.15 (1.05 to 1.26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Charlson Comorbidity Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(per point increase)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>No Immunosuppression</td>
<td>2.12 (1.59 to 2.81)</td>
<td>&lt;0.001</td>
<td>2.08 (1.55 to 2.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prednisolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other drugs</td>
<td>2.04 (1.52 to 2.72)</td>
<td>&lt;0.001</td>
<td>2.21 (1.65 to 2.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inhaled corticosteroids (ICS)</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>No ICS</td>
<td>0.67 (0.48 to 0.92)</td>
<td>0.013</td>
<td>0.74 (0.54 to 1.00)</td>
<td>0.049</td>
</tr>
<tr>
<td>ICS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1 (per 1 litre increase)</td>
<td>0.99 (0.85 to 1.15)</td>
<td>0.905</td>
<td>1.00 (0.86 to 1.17)</td>
<td>0.951</td>
</tr>
<tr>
<td>Anti-fibrotic drugs</td>
<td>1.54 (1.07 to 2.23)</td>
<td>0.02</td>
<td>1.38 (0.90 to 2.10)</td>
<td>0.149</td>
</tr>
</tbody>
</table>

A multivariate poisson regression model was used adjusting for age, sex, comorbidity by Charlson Comorbidity Index, ICS, immunosuppression, antifibrotic drugs. P values <0.05 were considered significant.

FEV1, forced expired volume in the first second; ICS, inhalation corticosteroids.
vaccination during the study period, leading to a selection bias of included patients and probably explaining at least in part the proportion of lost to follow-up in the cohort. Patients with incomplete data were similar to those with complete follow-up regarding baseline characteristics, besides a slightly higher proportion of males (data not shown) might affect the immunogenicity results. Second, although we matched patients and controls by sex and the nearest age, we could not match for age per-year increase, and healthcare personnel were younger and more often females. We conducted the analysis without matching, with similar results irrespective of whether matching or not was applied, but we cannot rule residual confounding completely out. Third, medical information from the controls was incomplete. Fourth, the duration of follow-up was not sufficient to assess waning long-term immunogenicity. Furthermore, even if we did not find a statistically significant difference between the time interval between the first and second doses, the time between the two doses was numerically slightly shorter for patients than for controls. However, the optimal time interval has been identified as 8 weeks, and since we observed only a few days shorter interval in the patients than in the controls, we do not suspect this could alter significantly the signal of our results. Finally, we did not have immunological markers of cellular immunity, which are also related to immunological protection after vaccination.

CONCLUSIONS
Most patients with chronic lung diseases could establish functional humoral responses to vaccination, but humoral responses were lower at 2 and 6 months in patients with chronic pulmonary diseases than those observed in controls. Age, comorbidities and the use of different immunosuppressants were all associated with impaired immunological responses.

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Contributors
All authors contributed to drafting the paper and revised the manuscript for important intellectual content. J-USJ, PS, ZBH, KKG, PG, SRO, HB, PG and SDN designed the study; ZBH, SRH, PS, SH, PK, TW, HP, DLM, LPA, CBH, MMP-H, AS, DLM, LDH, RBH, KF, JMM, JAA, RS-F, LDH, ES, AB, DLM, TD-S, RB-O and SDN contributed to data collection and analysis, ZBH, SRH, ASJ and PS verified the data and had full access to raw data. ZBH is the guarantor. All authors had full access to summary data reported in this study. All authors gave final approval of the version to be published.

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Disclaimer
The funding sources did not influence study design, data collection, analysis, or reporting of data.

Competing interests
None declared.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication
Not applicable.

Ethics approval
This study involves human participants and was approved by the Regional Scientific Ethics Committee of the Capital Region of Denmark approved the study (H-20079890). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data availability statement
Data are available on reasonable request. Data collected for this study, including individual participant data and a data dictionary defining each field in the set, can be made available to others in form of deidentified participant data. The study protocol and statistical analysis plan for the original study is available at www.cpttrin.dk. Informed consent forms will not be available according to Danish legislation. These data will become available from 1 August 2025, on reasonable request from investigators. Such requests, including study protocol with clear hypotheses, should be sent to the principal investigator, and the steering committee will review such a request. If the hypothesis does not comply with the informed consent supplied by the participants, and the hypothesis is judged to be valid, a data transfer agreement will be prepared, after which the data will be transferred. If the hypothesis is not covered by the given informed consent, the steering committee will assist in preparing an application for dispensation to our ethics committee.

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