Cerebrospinal fluid test results and associations with subsequent mental disorders, neurological diseases, and CNS infections: A population-based cohort study

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ABSTRACT

Background: Cerebrospinal fluid (CSF) immune alterations have been associated with mental disorders, neurological disease, and CNS infections; however, comprehensive large-scale longitudinal CSF studies are lacking.

Methods: By using the Clinical Laboratory Information System (LABKA) Research Database in the Central Denmark Region (1994–2012), we included 15,030 individuals tested for CSF WBC, CSF/serum albumin ratio, IgG index, total protein, albumin, or IgG with follow-up for the risk of mental disorders, psychotropic prescriptions, neurological diseases, or CNS infections, estimated by Cox regression.

Results: Among individuals receiving a mental disorder diagnosis (N = 1,147) after a CSF test, 30.0% had an abnormal CSF test result, while for those with a neurological disease (N = 3,201), 39.9% had abnormal test results, and among individuals with CNS infections (N = 1,276), 73.0% had abnormal test results. Individuals with abnormal CSF test results had an increased risk of mental disorders (HR = 3.20; 95%CI = 2.86-3.59), neurological diseases (HR = 12.40; 95%CI = 11.65-13.20), and CNS infections (HR = 338.59; 95%CI = 299-06-383.35) compared to individuals not registered with a CSF test. However, the risk of mental disorders was higher (P < 0.001) after CSF test results within the normal range (HR = 4.45; 95%CI = 4.08-4.86), whereas for neurological diseases (HR = 9.72; 95%CI = 9.19-10.29) and CNS infections (HR = 55.17; 95%CI = 47-12.64-60), the risk was highest after abnormal CSF test results (all P < 0.001). The risk of organic mental disorders tended to be highest in individuals with abnormal CSF test results (HR = 19.30; 95%CI = 13-44-27-71) even though not significantly different from the risk in the group of individuals with CSF test results in the normal range (HR = 13-55; 95%CI = 9-36-19-60) (P ≥ 0.05). Abnormal CSF test results were associated with an elevated risk of psychotropic prescriptions (HR = 3.91; 95%CI = 3-66-4-18), as were CSF test results within the normal range (HR = 4.26; 95%CI = 4-03-4-51) (P < 0.05).

Conclusions: Immunological CSF abnormalities are associated with an increased risk of mental disorders, neurological disease, and particularly CNS infections; however, the included CSF parameters were not specific for mental disorders and the relevant CSF biomarkers in psychiatry are yet to be discovered.
1. Introduction

Cerebrospinal fluid (CSF) analyses are the gold standard for diagnosing neuroinflammation and are primarily used when diagnosing neurological diseases and CNS infections. Increasing evidence suggests that immunopathological mechanisms play a role in the development of mental disorders in subgroups of individuals; however, current evidence is sparse regarding the prevalence of immunological CSF alterations in relation to mental disorders. Nonetheless, epidemiological studies have linked infections and autoimmune diseases with the development of mental disorders (Benros et al., 2013, Benros et al., 2011), genetic studies have shown associations with immune-related genes (Ripke et al., 2014; Wray et al., 2018), and meta-analyses of clinical studies have shown increased levels of peripheral proinflammatory markers across a broad range of mental disorders (Goldsmith et al., 2016; Köhler et al., 2017). Moreover, a potential immunological contribution is supported by the findings of beneficial effects of anti-inflammatory treatment on symptoms of depression (Köhler-Forsberg et al., 2019; Köhler et al., 2014) and psychosis (Jeppesen et al., 2020). However, immune components would likely need to reach the brain to be pathological regarding the development of mental disorders.

A recent systematic review and meta-analysis of all CSF studies of severe mental disorders (Orlovska-Waast et al., 2019) revealed that high-quality studies with relevant control groups are lacking, and only a few studies investigated the same parameters. Nonetheless, the meta-analysis found increased levels of CSF total protein and CSF/serum albumin ratio (albumin ratio) in schizophrenia and depression compared to healthy controls indicating an increased blood–brain barrier (BBB) permeability. Furthermore, increased CSF levels of IL-6, IL-8, and CSF/serum IgG ratio were found in schizophrenia (Orlovska-Waast et al., 2019). The meta-analysis did not find increased CSF WBC and IgG index (Orlovska-Waast et al., 2019); however, smaller studies either without controls or with only neurological controls did find increased levels of CSF WBC (Endres et al., 2020b, 2015; Oviedo-Salcedo et al., 2018; Pazzaglia et al., 1995; Schuld et al., 2004; Stich et al., 2015) and IgG index (Endres et al., 2020a; Kirch et al., 1985; Stich et al., 2015) in subgroups with psychotic and affective disorders. Also, some previous studies found an association between the clinical characteristics of patients with psychotic disorders and specific CSF findings, where an association with negative symptoms of schizophrenia and abnormal CSF findings have been found (Müller and Ackenheil, 1995), while others did not confirm this association (Rattay et al., 2021). Most prior CSF studies of mental disorders are limited by small case numbers and lack of longitudinal follow-up, control group, or adjustment of relevant confounders. Also, to the best of our knowledge, the association between specific CSF parameters and diagnoses of neurological disease and CNS infections has not yet been investigated in a large-scale longitudinal study.

We utilized the large population-based Danish longitudinal registers to assess routine CSF test results and investigate the association with subsequent mental disorders after both abnormal CSF test results and test results within the normal range. As comparison groups, we used the background population who did not undergo a lumbar puncture and individuals with neurological disease or CNS infection diagnoses. The hypothesis of the study was that immunological alterations are associated with mental disorders in a subgroup of psychiatric patients. To our knowledge, this is the largest study to date investigating the association between specific CSF inflammatory markers and subsequent diagnoses of mental disorders, neurological disease, and CNS infections.

2. Methods and materials

This study was based on linkage between Danish databases covering the Central Denmark Region and nationwide registers using the unique civil registration number assigned to every Danish resident.

2.1. Study population

All Danish residents born in Denmark and living or tested in the Central Denmark Region at some point during the period of 1994–2012 were included in our analysis (see flowchart in Fig. S1). In total, the cohort consisted of 214,997 individuals born on or after January 1, 1956, who were followed from January 1, 1994, until death, emigration, or study end on December 31, 2013. The linkage between the various registers was possible due to the Danish Civil Registration System, which contains demographic and other variables since 1968 (Pedersen, 2011). Individuals were followed in the Clinical Laboratory Information System (LABKA) Research Database (Grann et al., 2011) for the registration of a CSF test result and further in the Psychiatric Central Register (Mors et al., 2011) for a diagnosis of a mental disorder and in the National Hospital Register (Lyne et al., 2011) for diagnoses of neurological diseases or CNS infections in the follow-up period of January 1, 1994, to December 31, 2013. Individuals had to be minimum 1 year of age at some point during follow-up, i.e. individuals had to be born before or on December 31, 2012. Diagnoses in the National Hospital Register and in the Psychiatric Central Research Register were defined in accordance with the diagnostic system of the International Classification of Diseases Eighth Revision (ICD-8) until January 1, 1994. From hereafter, ICD-10 codes were used.

2.2. Exposure: Assessment of CSF test results

The LABKA database records results from analyses of specimens sent in by hospitals and general practitioners. The database has since the 1990’s covered the Central Denmark Region with complete coverage 2000–2012. Information in the register includes the test name, NPU (Nomenclature for Properties and Units) code and/or a local analysis number, test result, and measuring unit. We identified CSF test results for WBC (including mononuclear and polynuclear cell count), albumin ratio, IgG index, total protein, albumin, and IgG during the study period (NPV codes, analysis numbers, and reference intervals are listed in Table S1).

2.3. Outcome: Assessment of mental disorders, psychotropic medication, neurological diseases, and CNS infections

From the Psychiatric Central Register, we retrieved diagnoses of any mental disorder and the following categories: schizophrenia spectrum, affective, and organic mental disorders (see diagnostic codes in Table S2). The Psychiatric Central Register contains information on psychiatric inpatient contacts in Danish hospitals since 1969 and outpatient and emergency room contacts since 1995. The Danish National Prescription Registry (Wallach Kildemoes et al., 2011) provided information on first redeemed prescriptions after the CSF test of the following psychotropics (with Anatomical Therapeutic Chemical codes): antipsychotics (N05A), anxiolytics (N05B), and antidepressants (N06A). All-cause mortality was retrieved from the Danish Civil Registration System, and psychiatric hospital readmissions following the initial psychiatric diagnosis from the Psychiatric Central Register; both were used as proxy measures for treatment response. In posthoc analyses, we included outcomes of respectively neurological diagnoses (excluding CNS infections and stroke) and CNS infections to examine the exposure variables’ specificity.

2.4. Statistical analyses

Statistical analyses were conducted in Stata, version 15, and hazard ratios (HRs) were estimated by Cox regression. P < 0.05 was considered significant. The CSF variables were time-dependent, and with a hierarchical set-up, i.e., individuals with ≥ 1 CSF test with abnormal test results always counted in this group. Abnormal CSF test results were defined as results with values above the reference interval. The reference
group consisted of individuals resident in the Central Denmark Region 1994–2012 who were not registered with a CSF test. Individuals with psychiatric diagnoses/reeominated prescriptions before 1994 or before a CSF test were excluded. Individuals with tests with CSF erythrocytes >500 were excluded. Main outcomes were first-time diagnoses of mental disorders (both main and sub-diagnoses were included) and first-reominated psychotropic prescriptions. The basic adjustment model included birth year, sex, and calendar year. The full adjustment model further included the Charlson Comorbidity Index (CCI) score (Charlson et al., 1987), parental mental disorders, and maternal educational level (measured as highest attained educational level, grouped in basic (compulsory school), short (vocational training/high school), medium (up to and including bachelor) or higher education (graduate/postgraduate degree)). Since data on maternal education before 1969 is insufficient, adjustment for those born before 1969 was for the educational level in the index person. The CCI score was considered time-dependent. The CSF variables were not normally distributed; thus, the median instead of mean CSF levels was compared between individuals with/without outcome after the CSF test. Since the median is a personally identifiable value, an approximation of the median was calculated by back-transformation of each variable’s log-transformed mean CSF level. The risk of outcome was investigated in age groups and according to time since the test. Hospital admissions at a psychiatric hospital occurring ≥7 days after the first psychiatric contact (discharge date for inpatients and contact date for outpatients) were defined as psychiatric hospital readmission.

All data are available from Statistics Denmark to all researchers based in Denmark upon approval and regulation by the Danish Data Protection Agency.

2.5. Sensitivity analyses

In a sensitivity analysis with mental disorders as the outcome, we excluded individuals with neurological diagnoses or CNS infections before the CSF test and censored individuals with these diagnoses after the test since some of the CSF test results will be increased due to these diseases.

3. Results

During the study period, a total of 15,030 individuals with a CSF test conducted in the Central Denmark Region were included in our study, of which 5,768 individuals (38.4%) had abnormal CSF test results. After the performance of a CSF test, a total of 1,147 individuals were diagnosed with any mental disorder, of whom 344 (30.0%) individuals were registered with an abnormal CSF test result. Likewise, subsequent to a CSF test, 3,201 individuals were diagnosed with a neurological disease, of whom 1,276 (39.9%) individuals were registered with an abnormal CSF result. Moreover, 1,328 individuals were diagnosed with a CNS infection after a CSF test in the period, of whom 969 (73.0%) individuals were registered with an abnormal CSF test result. Baseline values for individuals with abnormal CSF test results, individuals with CSF test results in the normal range, and for the reference group of individuals without a CSF test is shown in Table S3.

3.1. Median of the CSF exposure variables

All medians (except IgG index) of the CSF variables were higher in the group of individuals with compared to without a CNS infection diagnosis after a CSF test (P < 0.001) (Table 1). For any mental disorder, most medians of CSF variables were higher in the group without mental disorders compared to the group with mental disorders after the CSF test (P < 0.001 or P < 0.001). However, there were no longer statistical differences between the groups regarding most CSF variables when individuals with neurological diagnoses were excluded from the analyses (Table S11). For neurological disease, medians of all cell counts were

| Exposure variable (unit) | Mental disorder diagnosis after the CSF test | Neurological disease diagnosis after the CSF test | CNS infection diagnosis after the CSF test | No CSF infection diagnosis after the CSF test |
|-------------------------|--------------------------------------------|-----------------------------------------------|------------------------------------------|--------------------------------------------|
| WBC (x 10^6/L)          | 713 3.61 (3.19–4.09)                       | 9,289 5.02 (4.63–5.49)                        | 2,225 2.63 (2.51–3.04)                   | 5,933 3.32 (3.20–3.44)                   |
| Mononuclear cell count (x 10^6/L) | 61 56.25 (47.61–69.05)                      | 266 54.09 (44.86–63.50)                       | 47 27.66 (19.77–35.60)                   | 1,183 15.62 (12.29–19.79)              |
| Polynuclear cell count (x 10^6/L) | 59 44.87 (36.74–53.64)                      | 57 27.14 (19.27–35.35)                        | 205 3.59 (2.50–4.69)                     | 202 1.78 (1.00–2.52)                   |
| Albumin ratio (x 10^3)   | 451 5.18 (4.94–5.42)                        | 1,503 3.24 (3.18–3.32)                        | 433 12.13 (11.10–13.19)                  | 593 15.62 (14.57–16.67)               |
| IgG index                | 449 0.52 (0.51–0.53)                        | 5,392 0.56 (0.55–0.57)                        | 2,773 0.55 (0.54–0.56)                   | 6,024 0.56 (0.55–0.57)                 |
| Total protein (g/L)      | 1,026 0.33 (0.32–0.36)                      | 1,108 0.62 (0.60–0.65)                        | 195 0.08 (0.06–0.10)                     | 196 0.08 (0.06–0.10)                  |
| Albumin (g/L)            | 523 0.21 (0.19–0.22)                        | 6,002 0.66 (0.65–0.67)                        | 202 0.69 (0.68–0.70)                     | 6,024 0.66 (0.65–0.67)                 |
| P-values for the statistical difference between medians in respectively the group with and without the given outcome after the CSF test, adjusted for sex, age, and calendar year: * P-value < 0.05 (not significant); P-value < 0.01; P-value < 0.001.
highest in the group without a neurological diagnosis after the CSF test (P < 0.01 or P < 0.001) while the medians of IgG index and IgG were highest in the group with outcome (P < 0.001) (Table 1).

### 3.3. Primary analyses: WBC, albumin ratio, and IgG index

Increased levels of WBC were associated with an HR of 2.31 (95%CI = 2.00-2.67) for mental disorders, an HR of 8.26 (95%CI = 7.66-8.91) for neurological disease, and of 247.32 (95%CI = 221.20-276.53) for CNS infections (Table 2). We found a strong dose–response relationship between an increasing number of WBC and the risk of CNS infections (P < 0.001), and in particular, the HR of a CNS infection was increased more than 2,300 times (HR = 2,369.56; 95%CI = 2,008.41-2,795.66) in individuals with WBC greater than 500 (Table 3). Furthermore, increased levels of the albumin ratio were associated with an HR of 2 (Table 2 and Fig. 1). However, for the neurological diagnoses, the HR was highest in individuals with abnormal CSF test results (HR = 12.40; 95%CI = 11.65-13.20) compared to individuals with CSF test results in the normal range (HR = 9.72; 95%CI = 9.19-10.29) (P < 0.001). Also, the HR for CNS infections was increased by 338.59 (95%CI = 299.06-383.35) in individuals with abnormal CSF test results, which was clearly higher compared to the HR of 55-17 (95%CI = 47.12-64.60) in individuals with CSF test results within the normal range (P < 0.001).

### 3.4. Secondary analyses: Albumin, total protein, and IgG

Increased levels of albumin were associated with an increased HR of 2.47 (95%CI = 2.00-3.05) for mental disorders, an increased HR of 8.25 (95%CI = 7.48-9.10) for neurological diseases, and an increased HR of 22.84 (95%CI = 19.04-27.38) for CNS infections, compared with individuals without a CSF test (Table 2). Moreover, increased levels of total protein were associated with an HR of 3.05 (95%CI = 2.63-3.54) for mental disorders, an HR of 7.83 (95%CI = 7.21-8.50) for neurological disease, and the risk of CNS infections was associated with an HR of 152.59 (95%CI = 134.45-173.18). Increased levels of IgG also elevated the risk of all outcomes (any mental disorder: HR = 1.85; 95%CI = 1.40-2.43; neurological diagnoses: HR = 12.61; 95%CI = 11.34-14.03; and CNS infections: HR = 7.09; 95%CI = 5.48-9.18).

### 3.5. Time since the CSF test

The risk of all outcomes was highest the first month after an abnormal CSF test result with an HR of 16.03 (95%CI = 10.96-23.45) for mental disorders, an HR of 392.55 (95%CI = 363.73-423.66) for neurological disease, and a markedly elevated HR of 10,270.91 (95%CI = 9,079-19.11,619.06) for CNS infections (Table 4). The risk of all outcomes was also highest the first month after a CSF test result in the normal range (any mental disorder: HR = 22.23; 95%CI = 16.59-29.79; neurological disease: HR = 406.49; 95%CI = 380.09-434.72; and CNS infection: HR = 1,677.04; 95%CI = 1,420.49-1,979.92). The risk of any mental disorder was still significantly increased 8–13 years after the test, both for individuals with abnormal CSF test results (HR = 2.43; 95%CI = 1.89-3.12) and individuals with CSF test results in the normal range (HR = 2.60; 95%CI = 2.11-3.20).

### 3.6. Age at the CSF test

CSF tests performed in the age group of 19–58 years seemed to display the highest risk of mental disorders (Table S4). Abnormal CSF test results in the age group 19–58 years of age and CSF test results in the normal range in the age group 7–18 years displayed the highest risks of neurological diseases. For CNS infections, the risk was highest for the age group 7–18 years.

#### Table 2

| Groups according to result of CSF test | Any mental disorder | Neurological disease | CNS infections |
|--------------------------------------|---------------------|----------------------|----------------|
|                                      | HR     | 95%CI    | HR     | 95%CI    | HR     | 95%CI    |
| Persons without a CSF test (reference)|        |          |        |          |        |          |
| Persons with abnormal CSF test results|        |          |        |          |        |          |
| Increased CSF test results (primary exposures) |        |          |        |          |        |          |
| WBC, increased levels | 205    | 2.31     | 2.00-2.67 | 788    | 8.26     | 7.66-8.91 |
| Albumin ratio, increased levels | 53     | 2.47     | 1.88-3.24 | 257    | 8.17     | 7.20-9.27 |
| IgG index, increased levels | 45     | 1.62     | 1.20-2.18 | 413    | 24.36    | 22.06-26.93 |
| Increased CSF test results (secondary exposures) |        |          |        |          |        |          |
| Albumin, increased levels | 90     | 2.47     | 2.00-3.05 | 434    | 8.25     | 7.48-9.10 |
| Total protein, increased levels | 189    | 3.05     | 2.63-3.54 | 643    | 7.83     | 7.21-8.50 |
| IgG, increased levels | 52     | 1.85     | 1.40-2.43 | 370    | 12.61    | 11.34-14.03 |
| Persons with CSF test results within the normal range |        |          |        |          |        |          |
| CSF test results in the normal range (primary exposures) |        |          |        |          |        |          |
| WBC, in the normal range | 606    | 3.23     | 2.95-3.54 | 1,738  | 7.97     | 7.54-8.42 |
| Albumin ratio, in the normal range | 353    | 3.45     | 3.07-3.89 | 1,270  | 9.38     | 8.79-10.00 |
| IgG index, in the normal range | 421    | 3.62     | 3.24-4.03 | 1,293  | 7.57     | 7.11-8.07 |
| CSF test results in the normal range (secondary exposures) |        |          |        |          |        |          |
| Albumin, in the normal range | 474    | 3.39     | 3.06-3.76 | 1,662  | 9.60     | 9.07-10.17 |
| Total protein, in the normal range | 816    | 3.82     | 3.52-4.14 | 2,367  | 8.95     | 8.52-9.40 |
| IgG, in the normal range | 324    | 3.41     | 3.02-3.85 | 1,016  | 7.12     | 6.64-7.63 |

a Adjusted for sex, age, calendar year, CCI-index score, parental history of psychiatric disorders and maternal or personal educational level. P-values for the statistical difference between HRs in respectively the group with abnormal CSF tests and CSF tests in the normal range.

b P-value ≤ 0.05 (not significant); c P-value < 0.05; d P-value < 0.01; e P-value < 0.001. HR = hazard ratio.
3.7. CSF test results and specific mental disorder diagnoses

Abnormal CSF test results were associated with an elevated risk of schizophrenia spectrum disorders (HR = 4.01; 95%CI = 2.69-5.97), affective disorders (HR = 3.69; 95%CI = 3.09-4.41) and organic mental disorders (HR = 19.30; 95%CI = 13.44-27.71) (Table S6). These HRs were not significantly different from the HRs in the group of individuals with CSF test results in the normal range for schizophrenia (HR = 6.08; 95%CI = 4.51-8.21) and organic mental disorders (HR = 13.55; 95%CI = 9.36-19.60), but for affective disorders, the risk in this group was highest (HR = 5.31; 95%CI = 4.64-6.08) (P < 0.001). Further analyses for these outcomes are shown in Table S5-S9.

3.8. CSF test results and psychotropic medication

The HR of redeemed prescriptions for psychotropic medication was highest in the group of individuals with CSF test results in the normal range (HR = 4.26; 95%CI = 4.03-4.51) compared with the group with abnormal CSF test results (HR = 3.91; 95%CI = 3.66-4.18) (P < 0.05) (Table S6). Further analyses are shown in Table S5-S9.

3.9. CSF test results and the risk of psychiatric readmissions and all-cause mortality

In individuals with any mental disorder, there was no significant association between all-cause mortality and neither abnormal CSF test results nor CSF test results in the normal range (Table S10). Nonetheless, the risk of psychiatric hospital readmission in both individuals with any mental disorder (HR = 1.76; 95%CI = 1.40-2.23), schizophrenia spectrum disorders (HR = 3.94; 95%CI = 2.38-6.53), and affective disorders (HR = 2.19; 95%CI = 1.62-2.96) was increased in the group of individuals with abnormal CSF test results. This risk was similarly elevated in the group of individuals with CSF test results in the normal range.

3.10. Sensitivity analyses

The significantly higher HR for mental disorders in individuals with WBC levels in the normal range compared to individuals with abnormal

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**Fig. 1.** Risk of mental disorders, neurological disease, and CNS infections according to the CSF test result compared with individuals who did not have a CSF test*. a Adjusted for sex, age, calendar year, CCI-index score, parental history of psychiatric disorders, and maternal or personal educational level. The error bars indicate 95% confidence intervals.
results gave a more than 12-fold elevated risk of neurological diseases. However, the risk of mental disorders was even higher in individuals with minor changes compared to the main results (Table S11-S15). The type of CSF WBC and risk of mental disorders, neurological disease, and CNS infections is shown in Table 3.

| Groups according to result of CSF test | Any mental disorder | Neurological disease | CNS infections |
|---------------------------------------|---------------------|---------------------|----------------|
| N | HR | 95%CI | N | HR | 95%CI | N | HR | 95%CI |
| CSF WBC 0-1 × 10^9/L | 269 | 3.47 | 3.05-3.94 | 668 | 7.70 | 7.09-8.36 | 116 | 35.50 | 28.95-43.54 |
| CSF WBC 2-3 × 10^9/L | 260 | 3.58 | 3.14-4.07 | 715 | 9.40 | 8.67-10.19 | 142 | 43.06 | 35.63-52.05 |
| CSF WBC 4-5 × 10^9/L | 61 | 2.75 | 2.13-3.55 | 228 | 11.95 | 10.46-13.65 | 49 | 47.29 | 35.39-63.21 |
| CSF WBC 6-25 × 10^9/L | 97 | 2.73 | 2.22-3.35 | 372 | 12.17 | 10.94-13.53 | 187 | 106.64 | 89.77-126.68 |
| CSF WBC 26-100 × 10^9/L | 32 | 2.46 | 1.74-3.49 | 96 | 7.49 | 6.12-9.17 | 177 | 551.91 | 461.70-659.73 |
| CSF WBC 101-500 × 10^9/L | 31 | 2.13 | 1.50-3.04 | 85 | 5.07 | 4.08-6.30 | 287 | 1,475.91 | 1,263.49-1,724.03 |
| CSF WBC greater than 500 × 10^9/L | 23 | 2.29 | 1.52-3.45 | 61 | 5.62 | 4.35-7.26 | 225 | 2,369.56 | 2,084.41-2,795.66 |
| Persons without a CSF test (reference) | 6,789 | 1.00 | | | | | | |

Types of CSF WBC

- Type of CSF WBC
  - CSF WBC greater than 500 × 10^9/L
  - CSF WBC 26-100 × 10^9/L
  - CSF WBC 101-500 × 10^9/L
  - CSF WBC greater than 500 × 10^9/L

P-values for the statistical difference between HRs in respectively the group with abnormal CSF tests and CSF tests in the normal range: *P-value ≥ 0.05 (not significant); † P-value < 0.001.

4. Discussion

In this largest study to date on CSF test results related to mental disorders, neurological disease, and CNS infections, we found a more than 3-fold increased risk of mental disorders in individuals with abnormal CSF test results with an increased risk across all CSF parameters. However, the risk of mental disorders was even higher in individuals with CSF test results in the normal range. Abnormal CSF test results gave a more than 12-fold elevated risk of neurological diseases and a 338-fold elevated risk of CNS infection diagnoses, which were higher risks than for the group of CSF test results within the normal range. Results showed a strong dose-response relationship between increasing WBC levels and risk of CNS infections, as expected. The increased risk of subsequent mental disorders in individuals with abnormal CSF test results remained significantly elevated throughout the 13-year follow-up period after the CSF test.

4.1. Strengths and limitations

The large population-based cohort with 19 years of follow-up and virtually no loss to follow-up is a major strength of the study. To the best of our knowledge, this is the largest CSF study and the only study of our knowledge. However, major strengths of this study include a large population-based cohort with 19 years of follow-up and virtually no loss to follow-up is a major strength of the study. To the best of our knowledge, this is the largest CSF study and the only study investigating the association between specific CSF markers and outcomes of mental disorders, neurological disease, or CNS infections with between-group comparisons. Moreover, the longitudinal design allowed us to assess CSF test results in individuals without previous psychiatric, neurological, or CNS infection diagnoses or redeemed psychotropic medication. The design eliminated recall bias since information on CSF test and outcome was registered independently of each other.

Main limitations include that we did not have information on the indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by medication. The design eliminated recall bias since information on CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by medication. The design eliminated recall bias since information on CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture. As the CSF tests are requested by indication for the lumbar puncture.
clinical indication, a clinician bias might increase the risk of being diagnosed with mental disorders after CSF results within the normal range, instead of another outcome, since CSF findings in the normal range represent a lack of paraclinical signs of neurological disease or CNS infection. Thus, the results might be impacted by confounding by indication as the CSF tests were not conducted as a screening of patients but performed due to an indication. Also, we only had information on CSF tests conducted in the Central Denmark Region in the period 1994–2012. Hence it was not possible to determine if individuals had previous CSF tests or CSF tests conducted in another Danish region. However, exclusion/censoring of individuals with neurological diagnoses or CNS infections, which we had nationwide register information on, only had minor impact on the results. Since the registers contain information on hospital contact and all prescriptions made in Denmark, our findings might not capture individuals with less severe mental disorders not treated in hospital settings or pharmacologically. Also, it was not possible to include confounders as BMI (Juncal-Ruiz et al., 2018) and smoking that might have influenced the levels of inflammatory markers.

4.2. Possible pathophysiology contributing to the associations between abnormal CSF test results and subsequent increased risk of mental disorders

The increased risk of mental disorders across all abnormal CSF parameters in our study might indicate that neuroinflammation and other pathophysiological mechanisms play a role in the development of psychiatric symptoms in a subgroup of the patients. An increased albumin ratio, as a sign of a compromised BBB, elevated the risk of mental disorders. A compromised BBB, possibly as a result of an infection (Kim, 2008), might leave the brain more vulnerable to harmful substances from the periphery, such as circulating cytokines or brain-reactive autoantibodies, which in animal studies have been shown to induce neuropsychiatric symptoms (Kowal et al., 2004). Our findings are in line with a recent meta-analysis that found an elevated albumin ratio in individuals with schizophrenia and affective disorders compared with healthy controls (Orlovska-Waast et al., 2019). Furthermore, we found that an increased IgG index, an indicator of intrathecal production of antibodies, and elevated CSF WBC levels, also increased the risk of mental disorders. The increase in WBC could be due to an infection, and large-scale epidemiological studies have also linked both CNS and other infections with the development of schizophrenia spectrum (Benros et al., 2011; Pedersen et al., 2020) and affective disorders (Benros et al., 2013; Pedersen et al., 2020).

4.3. Individuals with CSF test results within the normal range

Individuals with CSF test results in the normal range also displayed elevated risks of mental disorders, neurological diseases, and CNS infections throughout the analyses, which could be due to confounding by indication; similar methodological challenges were seen in another study finding an increased risk of inflammatory bowel disease for stool samples positive for campylobacter bacteria, however, the risk was just as high for campylobacter-negative samples (Nielsen et al., 2019). In our study, the lumbar puncture was most likely performed to rule out organic CNS etiology of the psychiatric or neurological symptoms. Thus, our findings of a further increased risk of mental disorders after CSF test results in the normal range likely reflect the diagnostic process, where CSF results in the normal range together with the lack of neurological symptoms lead to a mental disorder diagnosis rather than a neurological diagnosis, supported by the 22-fold increased risk of mental disorders the first month after a CSF test result in the normal range. Moreover, neurological disorders and particularly CNS infections (Pedersen et al., 2020), which also served as comparison groups to the analyses for mental disorders, have been shown to increase the risk of mental disorders which might also to some extent explain the increased risk of mental disorders after a CSF test. However, the exclusion/censor of individuals with neurological diagnoses or CNS infections before and after the CSF test did not particularly change the risk estimates for mental disorders, besides from removing the difference in median WBCs between those with/without mental disorders after the CSF test, further demonstrating that abnormal test results are more likely to lead to neurological rather than psychiatric diagnoses. Our results indicate that the included CSF parameters are unspecific for diagnosing mental disorders and that the relevant CSF biomarkers in psychiatry have not yet been discovered. Interestingly, the risk of organic mental disorders in those with abnormal CSF test results is increased 19-fold, which might be caused by neurological diseases or CNS infections, even though the risk was not significantly different from the risk for the group of CSF test results in the normal range. In line with this, a recent study found that CSF abnormalities were more often found in psychiatric patients given an organically based diagnosis of psychosis rather than patients given a schizophrenia spectrum disorder diagnosis (Rattay et al., 2021). Furthermore, even though the risk of neurological disease was highest after abnormal CSF test results, the included CSF parameters are possibly also somewhat unspecific for predicting neurological disease since only a few CSF parameters were highest in the group with abnormal test results. This finding could also reflect the broadened indication for lumbar puncture due to the increasing amount of diagnostic CSF biomarkers (Costerus et al., 2018) where the routine CSF parameters might be in the normal range, e.g., in the case of presence of CSF tau and beta-amyloid in dementia diagnostics. Nonetheless, the risk of neurological disease was 24-fold increased after an increased IgG index, possibly due to multiple sclerosis. Moreover, the included CSF parameters were highly specific for predicting CNS infection diagnoses, which is also seen in the strong dose–response relationship for increasing WBC levels. Noteworthy, CSF test results in the normal range increased the risk of CNS infections 55-fold, and the risk in the first month after the test was elevated more than 1,600-fold, possibly explained by increased levels of specific infection-related CSF parameters not included in our analyses.

4.4. Conclusion and perspectives

In this largest population-based study to date, our results indicate that signs of neuroinflammation and increased BBB permeability increase the risk of mental disorders more than 3-fold supporting the hypothesis of immunological mechanisms playing a role in a subgroup of individuals with mental disorders. However, individuals with CSF test results in the normal range had a higher risk of mental disorders suggesting that the included CSF parameters are unspecific for diagnosing mental disorders and primarily diagnostic for neurological disease and mostly for CNS infections. Future clinical studies are needed with discovery analysis of potential CSF biomarkers in individuals with mental disorders to improve the diagnostics in psychiatry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Stich, O., Andres, T.A., Gross, C.M., Gerber, S.I., Rauer, S., Langosch, J.M., 2015. An observational study of inflammation in the central nervous system in patients with bipolar disorder. Bipolar Disord. 17, 291–302. https://doi.org/10.1111/bdi.12244.

Wallach Kildemoes, H., Toft Sorensen, H., Hallas, J., 2011. The danish national prescription registry. Scand. J. Public Health 39, 38–41. https://doi.org/10.1177/1403494810394717.

Wray, N.R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E.M., Abdellaoui, A., Adams, M.J., Agerbo, E., Air, T.M., Andlauer, T.M.F., Bacanu, S.A., Bankhead-Cannon, M., Beekman, A.F.T., Bigdeli, T.B., Binder, E.B., Blackwood, D.R.H., Bryois, J., Buttenschon, H.N., Bybjerg-Grauholm, J., Cai, N., Castelao, E., Christensen, J.H., Clarke, T.E., Coleman, J.I.R., Colodro-Conde, L., Couvy-Duchesne, B., Craddock, N., Crawford, G.E., Crowley, C.A., Dashti, H.S., Davies, G., Deary, I.J., Degenhardt, F., Derks, E.M., Dhur, N., Dolan, C.V., Dunn, E.C., Eley, T.C., Eriksson, N., Escott-Price, V., Knadler, F.H.F., Finucane, H.K., Forstner, A.J., Frank, J., Gaspar, H.A., Gill, M., Giusti-Rodríguez, P., Goes, F.S., Gordon, S.D., Grove, J., Halt, I.S., Hannon, E., Hansen, C.S., Hansen, T.F., Herms, S., Hickie, I.B., Hoffmann, P., Homuth, G., Horn, C., Hottenga, J.J., Hougaard, D.M., Hu, M., Hyde, C.I., Iagnac, M., Jansen, R., Jin, F., Jorgenson, E., Knowles, J.A., Kohn, K., Kretzschmar, W.W., Krogh, J., Kutilik, Z., Lane, J.M., Li, Yihan, Li, Yun, Lind, P.A., Liu, X., Lu, L., Macintyre, D.J., MacKinnon, D.F., Maier, R.M., Maier, W., Marchini, J., Mbarak, H., McGrath, P., McGiffin, P., Medland, S.E., Mehta, D., Middeldorp, C.M., Mihailov, E., Milaneschi, Y., Milani, L., Mill, J., Mondimore, F.M., Montgomery, G.W., Mostafavi, S., Mullins, N., Nauck, M., Ng, B., Nivard, M.G., Nyholt, D.R., O’Reilly, P.F., Oskarsson, H., Owen, M.J., Painter, J.N., Pedersen, C.B., Pedersen, M.G., Petersen, R.E., Petersson, E., Peyrot, W.J., Piirt, G., Prithum, D., Purcell, S.M., Quoniz, J.A., Quist, P., Rice, J.P., Riley, B.P., Rivera, M., Saeed Mirza, S., Saxena, R., Schoevers, R., Schulte, E.C., Shen, L., Shi, J., Shyn, S.I., Sigurdsson, E., Sinnamon, G.B.C., Smit, J.H., Smith, D.J., Stefansson, H., Steinberg, S., Stockmeier, C.A., Streit, F., Stromma, J., Tansey, K.E., Teismann, H., Teumer, A., Thompson, W., Thomson, P.A., Thorsteinsen, T.E., Tian, C., Traylor, M., Treutlein, J., Trubetskoy, V., Uitterlinden, A.G., Umbricht, D., Van Der Auwera, S., Van Hemert, A.M., Viktorin, A., Visscher, P.M., Wang, Y., Webb, B.T., Weinsheimer, S.M., Weillmann, J., Willemsen, G., Witt, S.H., Wu, Y., Xi, H.S., Yang, J., Zhang, F., Aroz, V., Baune, B.T., Berger, K., Boomsma, D.I., Chichon, S., Dannloski, U., De Geus, E.C.J., Depaulo, J.R., Domenici, E., Domschke, K., Esco, T., Grabe, H.J., Hamilton, S.P., Hayward, C., Heath, A.C., Hinds, D.A., Kendler, K.S., Kluiber, S., Lewis, G., Li, Q.S., Lucas, S., Madden, P.F.A., Magnusson, P.K., Martin, N., McIntosh, A.M., Metaplu, A., Mors, O., Mortensen, P.B., Müller-Mulns, B., Nordestofo, M., Nothen, M.M., O’Donovan, M.C., Paciga, S.A., Pedersen, N.L., Pennings, B.W.H.J., Perles, R.H., Porteous, D.J., Potash, J.B., Prestig, M., Rieteschel, M., Schafer, C., Schulze, T.G., Smolders, J.W., Stefan, H., Teimeier, H., Uher, R., Volzke, H., Weismann, M.M., Wenge, T., Winslow, A.R., Lewis, C.M., Levinson, D.F., Breen, G., Borglum, A.D., Sullivan, P.F., 2018. Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. Nat. Genet. 50, 666–681. 10.1038/s41588-018-0090-3.