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CHAPTER 7

General discussion and summary
GENERAL DISCUSSION

In this thesis, we investigated the activity of the innate immune system in HD as reflected in several inflammatory immune parameters. Also, we aimed to gain further insight into the neuropsychiatric symptoms in HD, in particular irritability. In addition, we investigated the association between inflammatory immune parameters on the one hand and measures of disease progression (including neuropsychiatric symptoms and cognitive decline) on the other. In aging, and in neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, (neuro) inflammation is associated with the loss of normal functioning. Investigation of inflammatory immune parameters of inflammation in relation to markers of disease progression may provide further insight into the development of symptoms of HD. Also, inflammatory immune parameters that reflect disease severity in HD may be used as biomarkers in research and clinical care. In HD, such biomarkers are important because they may help to detect whether or not a potential therapeutic intervention is beneficial [1, 2]. We expected to find a correlation between increased levels of pro-inflammatory cytokines and positive acute-phase proteins on the one hand and markers of disease progression on the other; we also expected that, with progressing disease, levels of regulatory anti-inflammatory cytokines would decrease. Finally, we also expected to find an association between levels of immune parameters and neuropsychiatric symptoms.

INTEGRATION OF THE STUDY FINDINGS INTO CURRENT KNOWLEDGE

Irritability

We assessed the psychometric properties of the Irritability Scale (IS) and established that a cut-off point of 14 provides a valid and reliable assessment of irritability in HD gene expansion carriers. Using the IS, in our HD population the prevalence of irritability in the past two weeks was 35%, whereas in other studies the prevalence ranged from 33-73% [3]. These differences in reported prevalence can probably be attributed to the wide range of instruments used to assess irritability, the different time periods of the studies, as well as the sociodemographic and clinical differences in the HD populations investigated. Currently, the IS the only symptom-specific scale that is recommended for the assessment of irritability in HD [4]. Although several scales are available that assess irritability as one of the behavioral domains (such as the UHDRS behavioral subscale), these subscales have either not been extensively validated [5] or no data are available on their reliability or other psychometric properties in HD [6-8].

In our study, the incidence of irritability was found to be 23% over a two-year period. The rate of remittance (for which we were unable to find any predictors) was comparable to the incidence of irritability over time. Increasing irritability was associated with increased apathy and the continuous use of antipsychotics. However, we found no correlation between irritability and measures of disease progression, such as cognitive decline, motor abnormalities and functional
incapacity. Also, at baseline, none of these parameters could predict the incidence of irritability over a two-year period. Earlier studies showed that irritability may be a characteristic neuropsychiatric symptom of pre-manifest and early HD[9-11]. This is in line with findings of more recent imaging studies that demonstrated functional changes in the brain of gene expansion carriers, specifically for the early stages of HD, that correlated with irritability[12, 13]. Possible explanations for the development of irritability include overcompensation of damaged circuits early in the disease[13] and cognitive overload caused by early cognitive decline[12]. Also, the construct of irritability as assimilated in the IS may lose validity in later stages of HD because of the interference of severe motor dysfunction, cognitive decline and neuropsychiatric symptoms.

**Immune parameters**

We found that activation of the acute-phase response, as reflected in levels of both positive and negative acute-phase proteins, was associated with impaired daily functioning, apathy and cognitive impairment. However, these associations were confounded by the use of antipsychotics. Gene expansion carriers that continuously used antipsychotics had higher levels of hsCRP and lower levels of albumin than gene expansion carriers who did not use antipsychotics during the study period. In other HD cohorts, CRP levels were significantly increased in advanced disease stages when compared to healthy controls; to our knowledge, in these cohorts, no adjustment was made for the use of antipsychotics. Levels of TNF-α and IL-10 increased over the two-year study period. Pre-motor symptomatic gene expansion carriers had lower levels of IL-5 and IL-6 than symptomatic gene expansion carriers. IL-6 and IL-8 were inversely associated with daily functioning. Also, increased levels of both IL-1ra and IL-6 were independently associated with impaired cognitive functioning. Previous cross-sectional studies with a limited number of mutation carriers in disease stages from premanifest to moderately advanced disease stage, presented evidence for increased immune activation in the plasma of HD mutation carriers[14-18]. Most consistently, levels of IL-6 were higher in motor symptomatic HD mutation carriers than in matched controls, whereas increased plasma levels of sIL-2R, sTNF-αR[18], IL-8, and TNF-α [14] were also found. The study that assessed IL-4, IL-5, and IL-10, found positive correlations with a more advanced disease stage, although levels of IL-4 and IL-5 were increased only in a moderately advanced disease stage, compared with controls[14].

The triggering of innate immune activation is also emerging in other neurodegenerative diseases[19]. However, the relationship between immune activation and disease progression is complex. In Alzheimer’s disease, inflammatory pathways are activated as reflected by increased levels of chemokines and cytokines in both the brain and cerebrospinal fluid of patients, and neuroinflammation has been demonstrated by PET scans[20]. On the other hand, inflammatory processes are also a risk factor for Alzheimer’s disease. Studies on patients with Alzheimer’s disease have demonstrated the negative effects of severe infection[21], sepsis[22] and periodontitis[23] on cognition.
By chance, we found evidence for activation of the acute-phase response in gene-expansion carriers who used antipsychotics. This was found when adjusting for the use of antipsychotics in the relationship between acute-phase proteins on the one hand, and daily functioning, cognitive impairment and apathy on the other. After this adjustment, effect sizes for these relations were considerably reduced and no longer significant. Furthermore, the group of gene-expansion carriers that continuously used antipsychotics had higher levels of CRP and lower levels of albumin than those who did not use antipsychotics. To our knowledge, this phenomenon has not earlier been described.

Antipsychotics are frequently prescribed in late-stage HD for the symptomatic treatment of movement disorder. Therefore, the idea that increased acute-phase response activity is at least partly due to the use of antipsychotics is in line with a study reporting that premanifest and early-HD gene-expansion carriers had similar lower CRP levels compared to controls[24]. In non-HD populations, two pathways for activation of the acute-phase response by antipsychotics have been described. First, low-grade inflammation may be the result of obesity[25], dyslipidemia[26] and insulin resistance[27] which are elements of the metabolic syndrome, a well-known side-effect of second-generation antipsychotics. Second, both classical antipsychotics[28] and second-generation antipsychotics[29] may directly induce low-grade inflammation through their hepatic impact.

**Strengths and limitations**

The strengths of this study are the cohort study design and the well-characterized study population with HD. Furthermore, we included a control group consisting of first-degree relatives without the HD gene expansion which had been exposed to a similar environment as the gene expansion carriers. Also, the group of gene-expansion carriers was heterogeneous, comprising a range of pre-manifest individuals many years from clinical onset to institutionalized patients with end-stage disease. In addition, the same individuals were interviewed up to three times, with a two-year interval in between. Specifically, we used valid and reliable measurement tools in a standardized setting. Dropout rates between measurements were moderate and did not exceed 20%. Cytokine levels were also measured in a standardized and state-of-the-art manner. Where possible, we used high-sensitivity enzyme-linked-immuno-sorbent assays (ELISA); due to their conceptual design, ELISAs are less prone to altered levels of circulating proteins and inhibitors than multiplexed immunoassays[30]. Also, the number of freeze-thaw cycles was limited to one (for IL1-b, IL-6, IL-10 and TNF-α) or two (for IL-1ra, IL-5 and IL-8). Third, the methodological design eliminated any plate-to-plate and/or lot-to-lot variation and/or operator variability, as paired samples from HD gene expansion carriers were measured simultaneously on the same plate for the longitudinal analyses. Fourth, significant partial correlations were found for all cytokines between paired HD samples, ranging from 0.483 to 0.728 (p<0.001).

Some limitations also need to be addressed. An important drawback is the limited validity of the measurement of behavioral symptoms in HD. Validated systems, such as the Diagnostic
and Statistical Manual of Mental Disorders (DSM), are used for the classification of psychiatric disorders. However, their usefulness in HD is limited because they were not designed to assess symptoms and do not assess the severity of symptoms in dimensional scales. Next, cytokine levels in the plasma may not accurately reflect the highly complex state of the immune system. Although blood and its subsequent handling was done carefully, we were not able to take into account the effect of diurnal and seasonal changes in cytokine levels, which can be significant[31]. Another drawback is that we could not adjust for all possible confounding variables. Medication is frequently prescribed for movement disorder and behavioral symptoms; these medications include antipsychotics and benzodiazepines, which are known to have a wide range of biological adverse effects. Given the observational nature of our study, we could not determine whether the associations found were explained by causative mechanisms, confounding, or confounding by indication. Given the low incidence of HD, collecting a sufficiently large sample of HD gene expansion carriers remains a challenge. In particular, for analyses within subgroups with a sizable number of covariates, sample sizes were too low to reach adequate statistical power; because this may have caused type-I and type-II errors, our findings need to be replicated in independent samples of HD gene-expansion carriers.

**Implications for clinical practice**

No specific treatment for irritability in HD is available. Current treatment guidelines are largely based on case series and expert opinion, and on findings from studies in patients with psychiatric or other neurodegenerative disorders[32-34]. Our study showed that, in a significant number of patients, irritability may remit over a two-year study period; however, we were unable to predict which patients had the highest chance to remit from irritability. Nonetheless, we found that the use of benzodiazepines and antipsychotics was associated with the prevalence and incidence of irritability, respectively. Both types of drugs can be used in the treatment of irritability; however, especially in patients who receive these drugs for other indications, clinicians should be aware that irritability might be a possible side-effect.

Although evidence for increased levels of (low-grade) systemic inflammation was found in our population, the cause of this systemic inflammation could not be identified; the inflammatory state most likely reflects a complex system of mechanisms. In other populations, the effects of systemic inflammation on mood[35-37] and cognition[38-40] are detrimental. Studies have also indicated negative effects on metabolic disease and cardiovascular health[41-43]. Several studies have investigated the positive effects of anti-inflammatory drugs in HD. However, until now, agents like minocycline[44, 45], cannabinoids[46, 47] and non-steroidal anti-inflammatory drugs[48, 49] have not proven effective in humans, although some studies may have been under-powered. Further studies are needed to assess whether a more specific tweaking of neuroinflammation may benefit the many ailments of HD gene expansion carriers. In HD, treatable causes of systemic inflammation, such as infectious processes, should be managed properly. Also, iatrogenic damage, caused by the possible adverse effects of prescribed drugs, should be prevented.
Although our findings offer additional insight into the role of the innate immune system in HD, they also indicate that the rather crude immune parameters that we investigated were not of clinical use as biomarkers for the individual HD gene expansion carrier. It is important that a biomarker is measurable in a cost-effective manner, changes with the disease state over time, and is able to discriminate between gene expansion carriers in different disease states.

**Directions for future research**

The studies in this thesis confirm that inflammatory processes play an important role in HD. Immune parameters, indicating activation of the innate immune system, correlated with neuropsychiatric and motor symptoms, as well as with functional decline. However, given the observational nature of our investigation, we cannot infer any causal relations. Most likely, the immune system plays both a pathogenic and homeostatic role; however, more longitudinal studies are required to unravel its precise function in HD. Importantly, our findings also demonstrated that iatrogenic factors, such as the use of (psychotropic) medication, were related to both immune system parameters and outcome measures and, thus, need to be taken into account.

Although we have provided a thorough assessment of the psychometric properties of the IS, these findings still need to be replicated in larger studies. The validity and comparability of the scale, and its sum score in different stages of the disease, should be studied further using more sophisticated analysis techniques that include confirmatory factor analysis to assess measurement invariance. This might help establish whether factor loadings, intercepts and residual variances are equivalent in a factor model that measures a latent variable irritability. If so, comparisons of irritability scores will be valid across disease stages and across time within a HD gene expansion carrier.

Our findings concerning the associations between immune system parameters on the one hand and neuropsychiatric symptoms and disease progression on the other, yielded far more modest effect sizes than other studies of a comparable size Therefore, larger studies are needed to replicate these findings. Moreover, these studies should be able to adequately assess cytokine levels, taking into account possible confounding factors such as diurnal and seasonal variations in the levels of cytokines and the use of medication.

In observational studies, no firm conclusions regarding causality can be drawn. In our study, the confounding effect of the use of antipsychotics on immune system parameters and neuropsychiatric symptoms, warrants investigating whether this is explained by confounding by indication or by a direct effect of these drugs. Therefore, the results of previous studies on antipsychotics cannot be generalized. The effectiveness of antipsychotics should be assessed critically in well-designed and sufficiently powered studies and be weighed against the possible negative consequences, preferably in randomized controlled trials (RCTs) in a HD population. In addition to evaluation of the safety of antipsychotics, RCTs can shed more light on the treatment of various neuropsychiatric symptoms which, so far, relies mostly on case reports and expert opinion.
SUMMARY

In a cohort of HD gene expansion carriers, we assessed neuropsychiatric symptoms and several inflammatory immune system parameters. The aim was to investigate neuropsychiatric symptoms, in particular irritability, in more detail and gain further insight into the activation of the innate immune system in HD, as well as its role in disease progression.

The irritability scale (IS) was used to assess irritability. This scale proved to be a reliable and valid instrument for the assessment of irritability in HD. Using a cut-off point of 14, the prevalence of irritability was 35% in our population of HD gene expansion carriers. Gene expansion carriers tended to underestimate their level of irritability when compared to reports of their caregivers or spouses. Being married/living together and a longer CAG expansion in the Htt-gene were independent correlates of self-reported irritability. The incidence of new-onset irritability over a two-year study period was 23% and coincided with an increase of apathy; during this period, irritability remitted in 30% of previously irritable gene expansion carriers.

Levels of the pro-inflammatory cytokines TNF-α and IL-10 increased over the two-year study period. Also, pre-motor symptomatic gene expansion carriers had lower levels of IL-5 and IL-6 than motor symptomatic gene expansion carriers. Additionally, levels of IL-6 and IL-8 were inversely associated with the level of daily functioning.

Activation of the acute-phase response, as reflected by higher levels of CRP and lower levels of albumin, was associated with impaired daily functioning, apathy and cognitive impairment. However, these associations were confounded by the use of antipsychotics.

A weak association was found between increased levels of both IL-1ra and IL-6 on the one hand and cognitive decline on the other. No associations were found between cytokine levels and apathy, irritability and depressive mood.
REFERENCES


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General discussion and summary


