



**SAHLGRENSKA ACADEMY**

## **Clinical association of brain-derived neurotrophic factor in rheumatoid arthritis**

Degree Project in Medicine

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## **Abstract**

Degree project in medicine: Clinical association of brain-derived neurotrophic factor in rheumatoid arthritis- Amanda Sundquist

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**Background:** Rheumatoid arthritis (RA) is an autoimmune disease that affects the joints, leading to pain, rigidity and loss of function. CD14<sup>+</sup> cells are involved in the immune response of RA. Brain-derived neurotrophic factor (BDNF) is a protein that is expressed in the central nervous system, but it is also produced by activated immune cells. It has been shown that inflammation reduces circulating BDNF levels and thus RA may have negative consequences on BDNF function.

**Aim:** To investigate if there is a link between BDNF production by CD14<sup>+</sup> cells and clinical features of RA.

**Method:** BDNF production by CD14<sup>+</sup> cells from peripheral blood was measured in 35 female RA patients and analysed regarding inflammation, pain and functional ability. BDNF was measured in supernatants of CD14<sup>+</sup> cell culture using an enzyme linked immunosorbent assay (ELISA). The patients with BDNF production levels below the mean level in our study population comprised the low BDNF group.

**Results:** The low BDNF group had more signs of clinical inflammation, as measured by swollen joint count ( $p=0.0031$ ) and tender joint count ( $p=0.023$ ) and higher disease activity than the high BDNF group ( $p=0.0053$ ). The low BDNF group had higher score on pain-VAS

( $p=0.013$ ) and tender joint count ( $p=0.023$ ). The low BDNF group had worse hand function ( $p=0.0019$ ) and lower hand strength ( $p=0.0013$ ) than the high BDNF group.

**Conclusion:** Low production of BDNF by CD14+ cells was associated with higher disease activity in patients with RA. Clinical inflammation decreased the production of BDNF by CD14+ cells. Low production of BDNF has a connection with pain and inferior hand function.

**Key words:** Rheumatoid arthritis, brain-derived neurotrophic factor, disease activity, pain, functional ability

## **Background**

### ***Rheumatoid arthritis***

Rheumatoid arthritis (RA) is an autoimmune disease that affects the joints, leading to pain, rigidity and loss of function in affected patients. RA is morphologically expressed as systemic inflammation when leukocytes invade joints and causes swelling and pain. If untreated, joint inflammation quickly progress into damage and functional disability. [1, 2] The most common expression of the disease is symmetrical polyarthritis, but some patients may develop disease manifestation in other organs, such as blood vessels, lungs and kidneys. [1, 3] Neurological symptoms are frequent in RA patients. [1, 4] These problems expressed in RA are all a result of immune dysregulation. The most common sign of the inflammatory process in RA patients is clinical synovitis. Increased erythrocyte sedimentation rate (ESR) and elevated levels of c-reactive protein (CRP) also occur in some patients as signs of inflammation, although not disease specific. [1]

A cell involved in the immune response of RA is monocytes. Monocytes and macrophages along with other members of the myeloid cell line express the receptor CD14. Hence CD14 can be used as a marker for monocytes. CD14 is a pattern recognition receptor that binds LPS and cooperates with toll-like receptors (TLR). [5, 6] Monocytes that express CD14 have a role in RA producing proinflammatory cytokines. Monocytes also differentiate into macrophages, a cell responsible for synovial inflammation. There is also evidence that CD14+ cells differentiate into osteoclasts, the cell responsible for bone erosion which is a big part of the pathogenesis of RA. [5] Populational surveys have repeatedly shown that RA patients score lower regarding quality of life than the general population. Rheumatoid arthritis is a disease with numerous symptoms such as pain and loss of daily function which severely impairs the quality of life in the affected patient. [1, 7]

Rheumatoid arthritis often limits the performance of activities and the daily function in the patient. Historically RA often lead to progressive disability, today more aggressive treatment and earlier diagnosis has reduced the number of cases leading to severe disability. [1, 8] The health assessment questionnaire (HAQ) is the golden standard for measuring functional status in adult patients with RA. It measures physical function that is one domain in health-related quality of life. Thus, HAQ can be used to measure quality of life. In RA HAQ is one the strongest predictors of long-term outcomes of RA. [9] In RA small joints, such as joints in the hand, are often affected by inflammation, leading to functional impairment in the hand. [10, 11] The disabilities of the arm, shoulder and hand measurement (DASH) is a tool for assessing the impairment and limitation of the upper extremity including hand. In RA patients, a strong correlation has been found between disease activity, hand function measured

by DASH and hand grip strength. Thus, hand strength also is a measurement related to disease activity and disability in RA patients. [10]

### ***BDNF***

Brain-derived neurotrophic factor (BDNF) is a protein that belongs to the neurotrophin family. BDNF is most known for its expression and important role in the central nervous system (CNS). In the brain, production of BDNF occurs in glia cells and neurons but also in dendrites. The initial BDNF transcript is pre-pro-BDNF form that is later cleaved to mature BDNF. Mature BDNF is secreted both by presynaptic and postsynaptic terminals of the cell, thus with different stimulations for secretion. [12] In the periphery, BDNF is produced by activated immune cells such as T-cells, B-cells and monocytes but can also be found in platelets. [13, 14] BDNF binds primarily to the tropomyosin receptor kinase B receptor (TrkB), but also binds to the p75 neurotrophin receptor (p75NTR) leading to activation of different intracellular pathways for signalling. [12] TrkB can be found in B-cells and Th1-cells as well as in smooth muscle cells, airway epithelial cells, cardiomyocytes and cancer cells. [15, 16]

### ***BDNF and the brain***

Neuronal maturation, synaptic plasticity and synapse formation are all processes involving BDNF, thus showing the importance of BDNF for the CNS. [12] BDNF production can be found in almost all brain regions. However, the function of BDNF varies depending on composition of the tissue in each brain region as well as stage of brain development. The effects that BDNF exerts on the brain and its function can be explained by BDNF's role in maintaining a balance between stimulating and inhibitory processes in brain development and synaptic plasticity by different signalling pathways. [17] BDNF has a role in pain processing in the central and peripheral nervous system, where nociceptor derived BDNF is involved in

inflammatory pain. BDNF is necessary for regulation of pain thresholds. [18] In a study done on rats that lack the BDNF gene, one found reduced thresholds for thermal hyperalgesia compared to healthy subjects. [18] When looking at the mechanisms for hyperalgesia, BDNF seems to be a part of the inducing of hyperalgesia by binding to TrkB in the dorsal root of the peripheral nervous system. Thus, the presence of BDNF in the peripheral nervous system may be associated with more pain. However, few studies are done on the impact of BDNF on pain modulation in humans. [19] In a study about BDNF's ability to cross the blood-brain barrier it was discovered that BDNF completely crossed the blood-brain barrier in an intact form. [20] Evidence from studies on animals and humans thus suggest that circulating BDNF is important for central functions in many CNS diseases such as mood disorders. [21, 22] In patients with RA, relationship between BDNF and depression has been studied. Recent results show that RA patients with depression have a lower level of BDNF in the peripheral blood than RA patients without depression. [23] It is unknown whether changes of BDNF in the brain mirrors changes in the peripheral BDNF in RA patients. [24]

#### *BDNF, inflammation and RA*

A recent study reported that patients with RA had higher levels of plasma BDNF compared to the healthy population. Also, in an experimental study on rats, it was discovered that adjuvant-induced arthritis (AIA) increased BDNF in serum and decreased BDNF in brain. This study suggests that serum BDNF is not a good marker for measuring BDNF produced in the brain, and that arthritis affects the expression of BDNF both in plasma and in the brain [24, 25] However, other studies suggest that inflammation decreases the level of BDNF and thus the levels of BDNF would be lower in patients with active RA. A reduction of BDNF is observed when pro-inflammatory cytokines are administered to rats. The same effect is

achieved by administrating lipopolysaccharides (LPS). Both pro-inflammatory cytokines and LPS play a role in inflammation. [26] There are also other findings indicating that inflammation may affect BDNF expression. High levels of BDNF in human serum correlate with lower expression of inflammatory markers in serum. [27] Macrophage activation may depend on BDNF synthesis and/or TrkB-activation and thus BDNF may have a role in the macrophage-dependent inflammation. [28] There is some evidence that BDNF and TrkB are upregulated in activated macrophages, indicating that BDNF regulates macrophage activity. [29] BDNF among other neurotrophins and their receptors can also be found in the synovial fluid of RA patients, supporting its possible involvement in arthritis. [30] Thus, finding the clinical association of BDNF in RA would be important for the individual patients well-being as well as for the medical field.

## **Aim**

The aim of this study is to investigate if BDNF produced by CD14+ cells is related to clinical signs of rheumatoid arthritis. This study will examine if there is any association between BDNF and disease activity, inflammatory parameters, pain, quality of life and functional ability. Our hypothesis is that higher inflammation results in low levels of BDNF and has a broad clinical impact in RA patients.

## **Material and methods**

### ***Study population***

In total, 35 female patients with RA disease were recruited in this study from the patient cohort at the Rheumatology Clinic of the Sahlgrenska University Hospital. The clinical assessments and blood sampling were done in one visit during October and November 2019.

All patients were similar in age. The characteristics of the study population are shown in Table 1.

### ***Clinical examination***

Tender points were measured using TP18 (tender points 18), that is a diagram for fibromyalgia. It is based on 18 standardized points to which pressure is applied during clinical examination. A score was then given on a scale from 0 to 18 based on how many of the standardized points were tender. [31]

To evaluate clinical inflammation in RA a clinical examination was done regarding both swollen and tender joints. The localization of the joints was documented on a standardized joint-figure and patients were given a score based on how many swollen respectively tender joints were found during the examination. This gave us swollen joint count and tender joint count. These scores were then applied to calculate disease activity score 28 (DAS28). DAS28 provides an index based on number of swollen joints, tender joints and ESR-value.

Furthermore, the patients were divided into groups depending on how active the RA inflammation was based on their DAS28 score. The groups were defined as remission ( $<2.6$ ), mild disease activity ( $<3.2$ ), moderate disease activity ( $<5.1$ ) and high disease activity ( $>5.1$ ). [32]

Hand strength was measured using Grippit device. The patient is encouraged to grip the instrument as hard as possible for 10 seconds. The instrument displays a result for max strength, mean strength and a final value after 10 seconds. The results are displayed in the unit Newton. One hand at the time is tested. [33]

### *Questionnaires*

The health assessment questionnaire (HAQ) is used to assess self-reported functional status in adults with arthritis, a score is given on a scale from 0-3. 0 means “without difficulty”, 1 “with partial difficulty”, 2 “very difficult or with help of aid or other person” and 3 “can’t do it”. Thus, a higher score indicates a worse functional status. [9] The questions in HAQ regard the patient’s ability to execute daily activities and the questions are divided into eight different domains. The HAQ-score is calculated by sum of the worst score in every domain divided by eight. The final score between 0 and 3 thus representing the averaging of the worst score in the eight domains. [34]

Fibromyalgia impact questionnaire (FIQ) is an instrument used to measure the effect of pain on functional status in patients with fibromyalgia and has a widespread clinical use. FIQ consist of three domains that are function, overall impact and symptoms. FIQ has one part with questions that are scored on a visual analog scale (VAS) and one can use these individual scores to measure the intended variable the question asses, The individual questions used were pain-VAS (Question 16), fatigue-VAS (Question 17) and depression-VAS (Question 18). FIQ can also be used to calculate a total score by using a standardized calculation method. The VAS-score is calculated as a number on a 100 mm scale [35].

The Disabilities of arm, shoulder and hand (DASH) questionnaire asses self-reported functional status of the upper limb. It can be used to detect disorders in the upper limb as well as changes over time. It also includes separate sections for work and sport/music for those who have jobs including a lot of upper limb involvement and those for who music and sport are an important part of their life. These sections are optional and are calculated separately and seldom used on RA patients. The DASH score includes in total 30 items. Scoring range

from 0, no difficulty to perform to 5, impossible to perform. The score is calculated as a sum of every answer. [36]

### ***Lab measurements***

C-reactive protein (CRP) was measured at the laboratory of Clinical Chemistry, Sahlgrenska University Hospital, using nephelometry (Beckman Immage 800). The cut off level for positivity was 5mg/l. Erythrocyte sedimentation rate (ESR) was measured at the laboratory of Clinical Chemistry, Sahlgrenska University Hospital, using the Westergrens method in where the sedimentation rate is measured as mm/h. The cut off level for positivity was 28 mm/h.

Platelet count and white blood cell count was measured at the laboratory of Clinical Chemistry, Sahlgrenska University Hospital, using particle count with optical measurement. The reference interval of platelet count is  $165 \times 10^9$ -  $387 \times 10^9$ /L. The reference interval for white blood cell count is  $3.5 \times 10^9$ -  $8.8 \times 10^9$ /L.

Haemoglobin (Hb) was measured at the laboratory of Clinical Chemistry, Sahlgrenska University Hospital, using photometry. The reference interval is 117-153 g/L.

Inflammation signature was calculated using CRP, ESR, white blood cell count, platelet count and Hb. Every abnormal parameter indicating inflammation, as defined above, is worth one point and the sum is calculated to give a score on a scale from 0 to 5, where a higher score indicated more laboratory signs of inflammation.

Anti-cyclic citrullinated peptides (Anti-CCP) and Rheumatoid factor (RF) were measured at the laboratory of Clinical Immunology at the Sahlgrenska university Hospital. For detecting Anti-CCP a multiplex method (anti-CCP2, BioRad, Hercules, CA) was used. The cut of level was 3.0 u/ml. RF was measured by rate nephelometric technology (Beckman image 800,

Beckman coulter AB, Brea, CA). The cut-off level was 20 U/ml. Both Anti-CCP and RF was taken from earlier patients records and was not measured at the time of the visit in 2019.

### ***BDNF and IFN- $\gamma$***

CD14<sup>+</sup> cells from peripheral blood were isolated and activated with LPS for 2h. BDNF produced by these cells was measured in the CD14<sup>+</sup> cell culture supernatant using an enzyme linked immunosorbent assay (ELISA) involving matched antibodies and recombinant standard (DY248, R&D Systems, Minneapolis, MN). The antibodies detect both pro-and mature BDNF. The detection limit was 15 pg/ml. The high BDNF production by CD14<sup>+</sup> cells in our study was set to 72.65 pg/ml corresponding to the average level in the study population.

Interferon-gamma (IFN- $\gamma$ ) was measured in CD14 cell culture supernatant using an ELISA involving matched antibodies and recombinant standard (M1933, Sanquin, Amsterdam, The Netherlands). The detection limit was 0.02 pg/ml.

### ***Statistical analysis***

The non-parametric Mann-Whitney U test was used on continuous data to analyse difference between the groups. Spearman correlation was used to analyse the correlation between parameters. Chi square test was used for comparison of prevalence between the high BDNF group and the low BDNF group. Descriptive values are presented as percentage or range [min-max]. GraphPad Prism version 8.3.1 was used for statistical analyses, Mann-Whitney U test and Pearson correlation. A p-value <0.05 was considered statistically significant. For Chi square test the site openepi.com was used. The box plot figures present data as median, and minimum to maximum values.

### **Ethics**

The study is approved by the Ethical Review Bord of Gothenburg, Dnr: 2019-03787, 2019-07-17 and is in line with the Declaration of Helsinki. Regarding taking part of information from patient records a confidentiality agreement has been signed by the operation manager. Patient data were handled according to current legislations in Sweden. Boibanc and privacy laws were followed. All patients in the study population have signed an agreement of consent.

## Results

### *Clinical and demographic characteristics of the study population*

Table 1. Clinical and demographic characteristics of RA patients

| Characteristics                             | n=35 |             |
|---|------|-------------|
| Females, n (%)                              | 35   | 100%        |
| Age, y                                      | 65   | [46-76]     |
| Disease duration, y                         | 13   | [0-45]      |
| Early RA, n (%)                             | 1    | 3%          |
| Established RA, n (%)                       | 14   | 40%         |
| Autoantibodies, n (%)                       | 28   | 80%         |
| RF+, n (%)                                  | 26   | 74%         |
| Anti-CCP+, n (%)                            | 18   | 51%         |
| Anti-CCP+ and RF+, n (%)                    | 16   | 46%         |
| DAS28                                       | 2.87 | [1.07-5.77] |
| High disease activity >5.1, n (%)           | 1    | 3%          |
| Moderate disease activity <5.1, n (%)       | 11   | 31%         |
| Low disease activity <3.2, n (%)            | 4    | 11%         |
| Remission <2.6, n (%)                       | 19   | 54%         |
| Swollen joint count, n                      | 1    | [0-8]       |
| Tender joint count, n                       | 3    | [0-14]      |
| ESR, mm/h                                   | 16   | [1-60]      |
| CRP, mg/ml                                  | 3    | [1-11]      |
| White blood cell count, x10 <sup>9</sup> /l | 6.2  | [3.4-12.9]  |
| IFN- $\gamma$ , pg/ml                       | 0.2  | [0.2-2.3]   |
| Right hand dominant, n (%)                  | 35   | 100%        |
| Methotrexate, n (%)                         | 23   | 66%         |
| TNF-inhibitors, n (%)                       | 10   | 29%         |
| Other biologics, n (%)                      | 6    | 17%         |
| Other DMARDs, n (%)                         | 2    | 6%          |
| No anti-rheumatic treatment, n (%)          | 6    | 17%         |
| Methotrexate+ other biologics, n (%)        | 4    | 11%         |
| Prednisolone, n (%)                         | 2    | 6%          |
| Treatment for depression, n (%)             | 6    | 17%         |

Absolute values are presented as mean [range]. Early RA: <3 years, Established RA: >10 years. DAS28: disease activity score 28. RA: rheumatoid arthritis, RF: rheumatoid factor, Anti-CCP: anti-cyclic citrullinated peptides, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, IFN- $\gamma$ : interferon-gamma TNF-i: tumor necrosis factor inhibitor, DMARDs: disease-modifying antirheumatic drugs

The study population of 35 patients were all female. The patients were around middle age, the age varied from 46-76 years old. The patients varied in disease duration between 0 and 45 years. Only one patient had early RA disease <3 years. 28 patients had autoantibodies RF

and/or Anti-CCP, 16 patients had a combination of RF+ and Anti-CCP+. One patient had high disease activity (DAS28 >5.1) and 54% of the patients were in remission (DAS28 <2.6). 23 patients were treated with methotrexate, and 4 patients were treated with a combination of methotrexate and other biologics. 6 patients had no anti-rheumatic treatment at all. There were 6 patients that had treatment for depression (Table 1).

### ***BDNF CD14+ Cells***

CD14+ cells were isolated from the peripheral blood and activated by LPS for 2h. BDNF released by CD14+ cells during the two hours of incubation was measured by the enzyme-linked immunosorbent assay as described above. First, absolute BDNF levels for each patient were

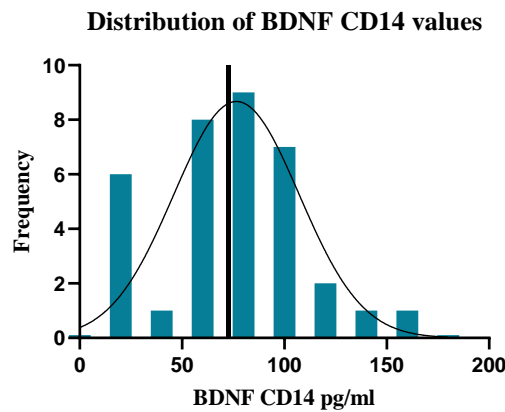


Figure 1. Distribution of BDNF levels in the patient cohort of 35 patients with rheumatoid arthritis. 15 patients had BDNF levels under the mean level and 20 patients above. BDNF: brain-derived neurotrophic factor

calculated according to the known concentration of recombinant BDNF and afterwards the mean BDNF level of the whole group was calculated. The patients with BDNF levels below the mean comprised the low BDNF group (n=15) and those above the mean BDNF level (n=20) were included in the high BDNF group. The BDNF measures in the study population was normally distributed around the mean (Figure 1). We performed comparison by using a non-parametric Mann-Whitney U-test that showed a significant difference between the two BDNF groups in BDNF levels. The other parameters that were analyzed were compared in relation to these BDNF CD14 groups.

### ***BDNF, disease activity and inflammation***

DAS28 is an instrument for measuring clinical inflammation and disease activity in RA. When calculating DAS28 one uses ESR, swollen joints and tender joints. The Mann-Whitney U test revealed that the group with low production of BDNF by CD14+ cells had a significant higher DAS28 compared to the group with high BDNF ( $p=0.0053$ , Figure 2A). This indicating that low production of BDNF increase the activity of the disease.

When looking at the parameters that constitute the DAS28 score, there was a significance difference in swollen joint count between the two BDNF-groups ( $p=0.0031$ , Figure 2B) and in tender joint count ( $p=0.023$ , Figure 2C) and not in ESR. Still, we observed a tendency to higher levels of ESR in the low BDNF group ( $p=0.15$ , Figure 2D). Taken together these results indicate that the

production of BDNF by CD14+ cells is lower in patients with high disease activity and in

patients with more clinical signs of inflammation. The difference in DAS28 found between

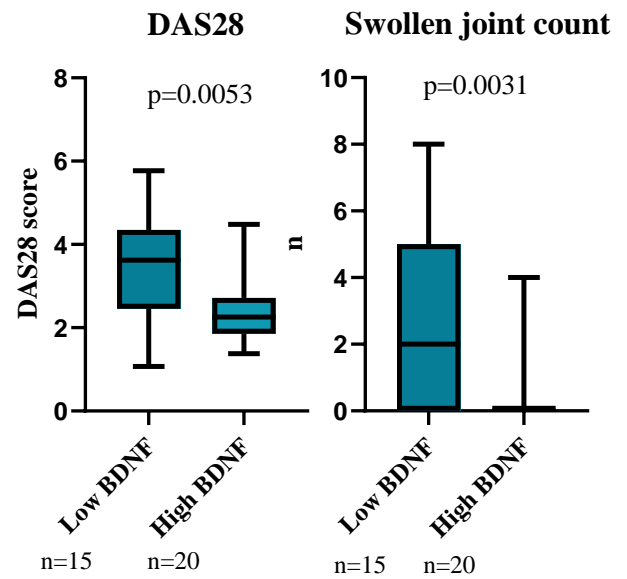


Figure 2A

Figure 2B

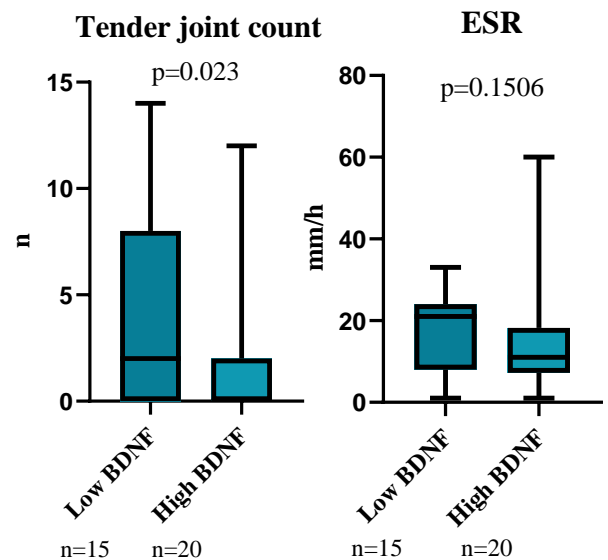


Figure 2C

Figure 2D

Figure 2A-D. Range of disease activity score 28, swollen joint count, tender joint count and ESR between the low BDNF group and the high BDNF group. BDNF: brain derived neurotrophic factor, ESR: erythrocyte sedimentation rate

the two BDNF groups in DAS28 depended on clinical signs of active joint disease, swollen and tender joints.

With regards to other measures of inflammation no significant differences were acquired.

Regarding white blood cell count, a low BDNF production was associated with higher white blood cell count ( $p=0.294$ ), although only four patients had leukocytosis ( $WBC > 8.8 \times 10^9/L$ ).

We did not find a difference in platelet count between the low and high BDNF group.

Regarding CRP, there was no difference between the groups. We also analyzed IFN- $\gamma$  in relation to BDNF, there was no difference in IFN- $\gamma$  production between the low and high BDNF group.

Table 2. Number of patients with active disease in the high BDNF group vs the low BDNF group

|                                   | High BDNF | Low BDNF |
|-----------------------------------|-----------|----------|
| Active disease, DAS28 >2.6, n (%) | 5 (25%)   | 11 (73%) |
| Total number of patients          | 20        | 15       |

DAS28: disease activity score 28, BDNF: brain derived neurotrophic factor

DAS28 score gives us the possibility to divide the patients into groups depending on their disease activity. Patients with DAS28 >2.6 have active disease. In the low BDNF group we found that 11 out of 15 patients had active disease, 73%. In the high BDNF only 25%, 5 out of 20 patients had active disease.

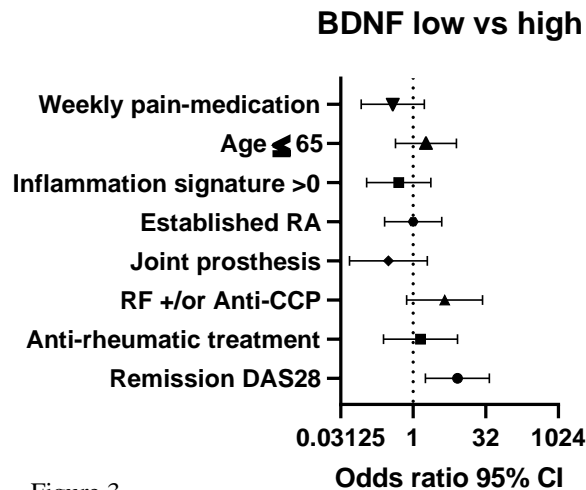


Figure 3

Figure 3. Odds ratio between the low BDNF group (n=15) and the high BDNF group (n=20). Odds ratio above 1 indicates that the characteristic is reported more frequently in the high BDNF group. Weekly pain-medication is defined as using pain-medication  $\geq 1$  time a week. Inflammation signature is a sum of ESR, CRP, white blood cell count, platelet count and Hb where every abnormal parameter is worth one point. Established RA is defined as a disease duration  $> 10$  years. Joint prosthesis is defined as having  $\geq 1$  joint prosthesis. RF+/or Anti-CCP is defined as having a measured positive value of these antibodies. Anti-rheumatic treatment is defined as having a treatment with methotrexate, DMARDS, TNF-i or other biologics. Remission DAS28 is defined as a score  $< 2.6$

BDNF: brain derived neurotrophic factor, RA: rheumatoid arthritis, RF: rheumatoid factor, Anti-CCP: anti-cyclic citrullinated peptides, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, Hb: haemoglobin, DMARDS: disease-modifying antirheumatic drugs, TNF-i: tumor necrosis factor inhibitor, DAS28: disease activity score 28, CI: confidence interval

### *Odds ratio low vs high BDNF*

When analyzed with a chi square test with a 95% confidence interval (CI) we found that low production of BDNF by CD14+ cells decreased the odds to be in remission ( $p=0.0064$ , Figure 3). There was no significant difference in production of BDNF between the group with no active anti-rheumatic treatment and the group that had rheumatic treatment ( $p=0.72$ , Figure 3). Regarding autoantibodies, no significant difference was observed between the BDNF groups. We found that having a low BDNF production in CD14+ cells lead to a decreased risk of having autoantibodies, although not significant ( $p=0.12$ , Figure 3). There was no significant difference in inflammation signature between the low and high BDNF group.

However, the results indicate having inflammation signature over 0 was somewhat more common in the group with low BDNF production ( $p=0.41$ , Figure 3). This indicating that the group with low production of BDNF in CD14+ cells may have more laboratory signs of inflammation. We found that there was no difference between the low and high BDNF group regarding having a disease duration >10 years, established RA ( $p=0.99$ , Figure 3). Regarding age there was no difference in age between the BDNF groups ( $p=0.44$ , Figure 3). There were no higher odds of having a joint prosthesis in the low BDNF group ( $p=0.24$ , Figure 3). There was no significant difference in using pain-medication on a weekly schedule between the low and high BDNF group ( $p=0.23$ , Figure 3).

### ***BDNF, pain and depression***

The group with low BDNF production by CD14+ cells scored significantly higher on pain-VAS ( $p=0.013$ , Figure 4). Pain-VAS is a part of FIQ-total that measures effect of pain on functional status. When analyzing the other parameters indicating pain such as tender joint count and TP18 there was a significant difference in tender joints ( $p=0.023$ , Figure 2C) but not in TP18 when it was analyzed separately ( $p=0.0765$ , Figure 5).

However, a tendency of higher TP18 was found in the low BDNF group.

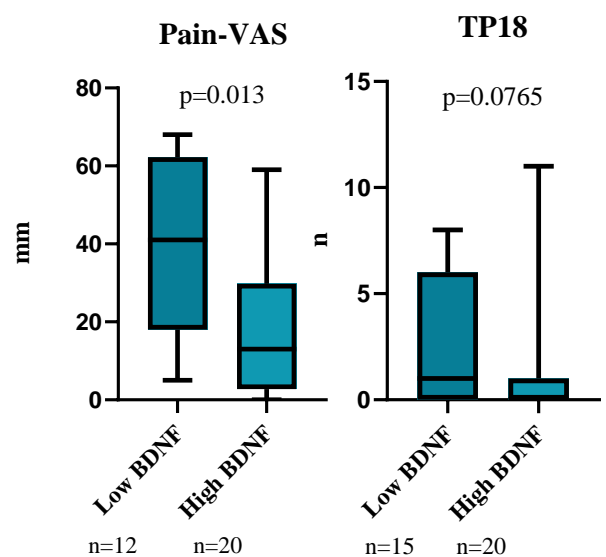


Figure 4

Figure 5

Figure 4-5. Range of mm on pain-VAS and number of TP18 between the low BDNF group and the high BDNF group.

VAS: visual analog scale, TP18: tender points 18, BDNF: brain derived neurotrophic factor

A low production of BDNF by CD14+ cells revealed a high significant score on FIQ-total ( $p=0.006$ , Figure 10). Other components in FIQ are fatigue-VAS and depression-VAS. We found a tendency of higher VAS-score on both these scales in the group with low production of BDNF by CD14+ cells ( $p=0.16$ , Figure 9) ( $p=0.065$ , Figure 10).

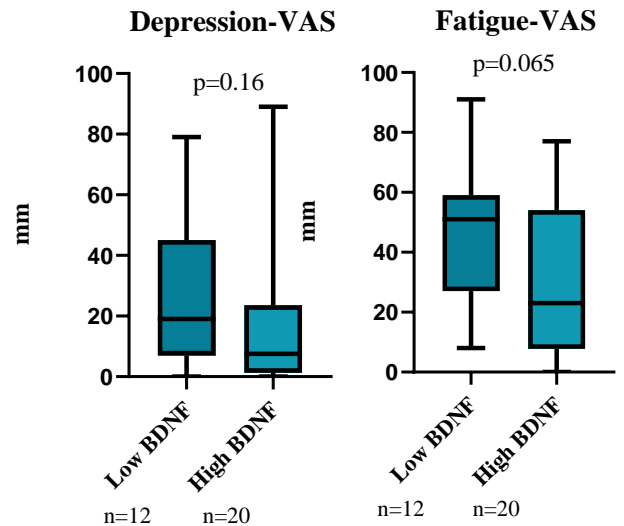


Figure 6

Figure 7

Figure 6-7. Range of mm on depression-VAS and fatigue-VAS between the low BDNF group and the high BDNF group.

VAS: visual analog scale, BDNF: brain derived neurotrophic factor

*Correlation between pain-parameters, BDNF in CD14+ cells and DAS28*

Pain-VAS had a negative correlation with BDNF ( $r=-0.35$ ,  $p=0.051$ , Figure 8). We also found a positive correlation between pain-VAS and DAS28 ( $r=0.49$ ,  $p=0.005$ , Figure 8), this indicating that pain may have a connection with disease activity in our study population. Pain-VAS had a correlation with TP18 and tender joint count (Figure 8). This indicating that pain-VAS has a connection with the other parameters used in this study for measuring pain.

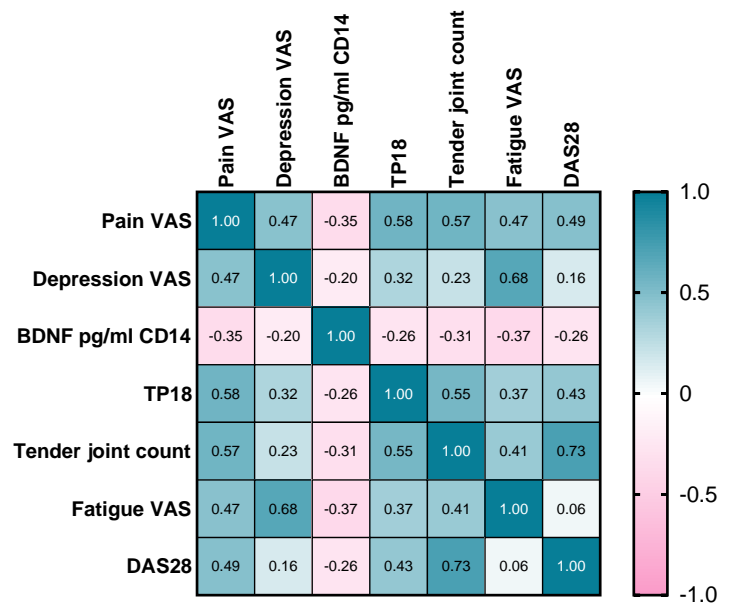


Figure 8. Correlation between parameters in the population of 35 RA patients. Correlation between BDNF produced by CD14+ cells, pain-parameters, depression, fatigue and DAS28.

VAS: visual analog scale. BDNF: brain-derived neurotrophic factor. TP18: tender points 18. DAS28: disease activity score 28. RA: rheumatoid arthritis

## *BDNF and functional ability*

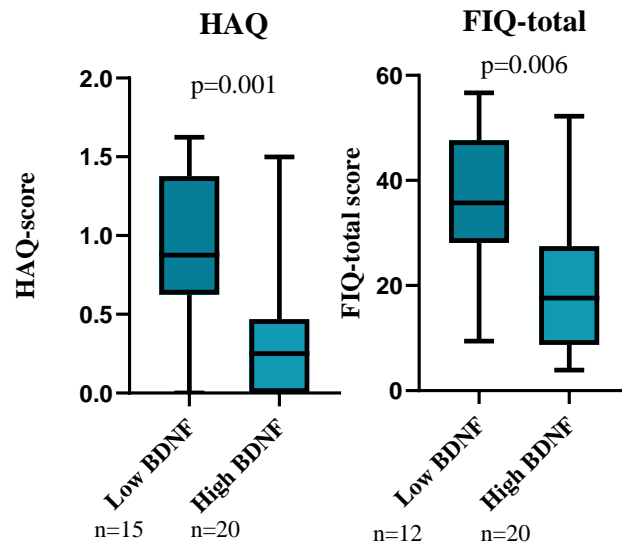


Figure 9

Figure 10

Figure 9-10. Range of HAQ-score and FIQ-total score between the low and high BDNF group.

HAQ: health assessment questionnaire. FIQ: fibromyalgia impact questionnaire. BDNF: brain-derived neurotrophic factor

HAQ is used to assess self-reported functional status in adults with arthritis, where a high score indicates a worse functional status and worse quality of life. In our study, low production of BDNF by CD14+ cells indicated a significant higher HAQ-score and thus a worse functional status and quality of life ( $p=0.001$ , Figure 9).

FIQ is an instrument which has been developed to measure effect of pain on functional status in patients with fibromyalgia. In RA patients, the results showed a significant difference where a low production of BDNF indicated a higher FIQ-total score and thus a worse functional status ( $p=0.006$ , Figure 10). Thus, both HAQ and FIQ were negatively related to BDNF production, low BDNF indicating worse functional status.

In the DASH questionnaire that was used to investigate activities related to function in arm/shoulder and hand in RA patients, we found that patients with a low production of BDNF by CD14+ cells scored higher on this questionnaire, indicating a worse arm/shoulder and hand health (p=0.0029, Figure 11). When looking at arm, shoulder and hand separately we found that there was a significant difference between the low BDNF and high BDNF group, where the low BDNF group scored higher regarding hand function (p=0.0019, Figure 12) as well as in function of the arm and shoulder (p=0.0056 respectively p=0.003, Figures not shown)

When looking further on hand function of RA patients, we found a significant difference in maximal hand strength on both the right and left hand. In this study, all patients were right hand dominant. Regarding hand strength, the low BDNF group had significantly lower hand strength in their dominant hand (p=0.0013, Figure 16). This together with the results in DASH-hand indicates a worse function of the hand in patients with a low production of BDNF by CD14+ cells.

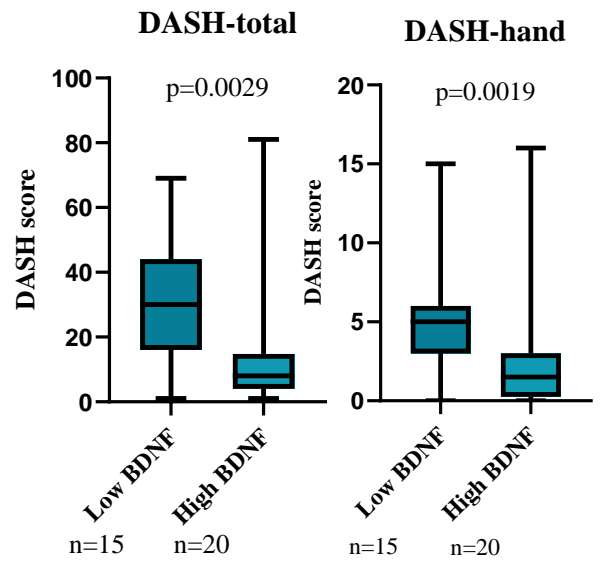


Figure 11

Figure 12

Figure 11-12. Range of score of DASH-total and DASH-hand between the low and high BDNF group.

DASH: disability of the arm, shoulder and hand questionnaire. BDNF: brain-derived neurotrophic factor

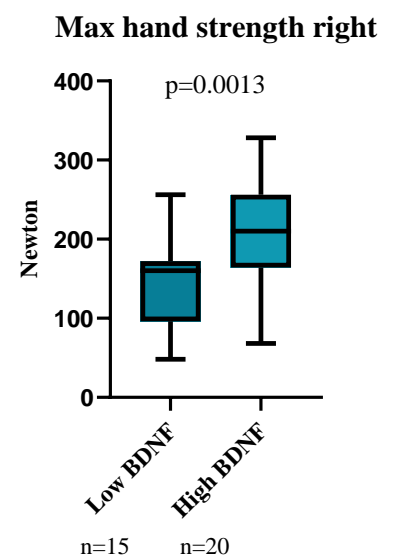


Figure 13

Figure 13. Range of max hand strength between the low and high BDNF group measured with Grippit.

BDNF: brain-derived neurotrophic factor

Both the low BDNF group and the high BDNF group had a median age of 67 years (Figure 17,  $p=0.29$ ). There was no significant correlation between age and maximal hand strength ( $r=-0.08$ ,  $p=0.66$ , Figure not shown). There was no significant difference between the group regarding disease duration. The low BDNF group had a median disease duration of 6 years and the high group a median disease duration of 9.5 years (Figure 18,  $p=0.76$ ). There was no correlation between disease duration and maximal hand strength ( $r=0.002$ ,  $p=0.99$ , Figure not shown).

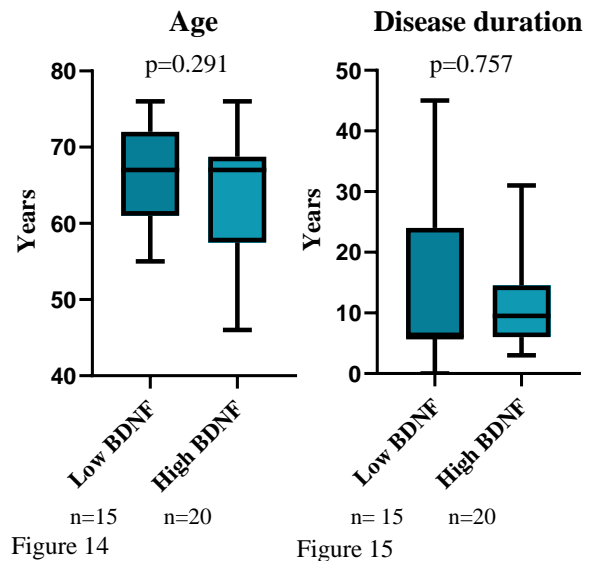


Figure 14-15. Range of years of age and years with disease between the low and high BDNF group.

BDNF: brain-derived neurotrophic factor

The results show that the group with low BDNF production has a worse overall functional status by HAQ and FIQ and in the upper extremities including hands.

## Discussion

The results of our study show that a low production of BDNF by CD14+ cells indicate higher disease activity scored by DAS28 and that clinical inflammation decreases the production of BDNF. Low production of BDNF by CD14+ cells also indicate more pain, worse quality of life and worse functional status overall. The hand strength and hand function are also inferior in the low BDNF group.

### ***BDNF, disease activity and inflammation***

The results showed that the group with low production of BDNF by CD14+ cells got a significantly higher DAS28 score, indicating higher disease activity. This increased DAS28 score in the group with low production of BDNF by CD14+ cells intriguingly depended mostly on more swollen and tender joints in our study population and not on ESR. However, both swollen and tender joint are indicators that can be used to measure inflammation. Thus, it seems that clinical inflammation reduces the production of BDNF by CD14+ monocytes. The result can be supported by findings from a study done on patients with unipolar depressive episodes where they showed that a high BDNF level in serum indicated lower expression of inflammatory markers in serum, such as TNF- $\alpha$  (tumor necrosis factor-  $\alpha$ ) [27] This meaning that higher levels of BDNF in serum indicate less active inflammation, in line with our results. We did not find a significant correlation between BDNF produced by CD14+ cells and BDNF in serum in our population. Thus, this together with the lack of studies on BDNF produced by CD14+ cells mean that we cannot draw certain conclusions from studies done on serum BDNF.

Regarding of other lab tests used to measure inflammation, no significant results were acquired. We only found a tendency of higher white blood cell count and ESR in the low BDNF group. When looking at inflammation signature that is a sum of ESR, CRP, white blood cell count, platelet count and Hb, one can say that there was a tendency of it being more common having an inflammation signature above zero in the low BDNF group. Inflammation induced by pro-inflammatory cytokines have been shown to reduce the expression of BDNF in rats. [26] The proinflammatory cytokine IFN- $\gamma$  has been found to reduce the BDNF expression in human bronchial cells [37]. However, another study implies that pro-

inflammatory cytokines, IL-6 (interleukin 6) and TNF- $\alpha$ , enhance BDNF secretion in peripheral monocytes. [38] Contradictory, in our study there was no significant difference between the low and high BDNF group regarding IFN- $\gamma$ . This may depend on the small study population and that many patients presented a value of zero regarding IFN- $\gamma$ . We did not analyse the relation between IL-6, TNF- $\alpha$  and BDNF produced by CD14+ cells. Thus, we did not find a strong connection between laboratory signs of inflammation and BDNF produced by CD14+ cells. The small study population and a low frequency of abnormal inflammatory test values in the study population may have a role in these results. For future studies, this relation needs to be wider explored and maybe then more evidence of a relationship between laboratory signs of inflammation and BDNF may be found.

When looking at the different disease activity groups divided from DAS28 score we found that there was a significantly higher odds of having low production of BDNF with active disease. A much higher percentage of the patients in the low BDNF group had active disease. This also supports that a low BDNF production by CD14+ cells from peripheral blood indicates higher disease activity. Further, the patients in our study had a mix of different treatments for their RA and some had no treatment at all. To get more certain results regarding inflammation and disease activity one should aim for a study population with the same rheumatological treatment. In conclusion our results indicate that clinical inflammation affects the production of BDNF, where having inflammation decreases the production of BDNF by CD14+ cells and where low production of BDNF indicate a higher disease activity.

### ***BDNF, pain and depression***

The group with low BDNF production by CD14+ cells scored significantly higher on pain-VAS indicating more pain in this group. When looking at TP18 and tender joint count, other

instruments used for measuring pain, trend of higher TP18 and higher tender joint count were found in the low BDNF group. Pain-VAS did correlate with DAS28. Inflammation has a known role in central pain in patients with RA. [39] The connection between pain-VAS and DAS28 supports that it may be inflammation and high disease activity rather than the low level of BDNF that causes the elevated pain perception in the low BDNF group. DAS28 also has a significant correlation to tender joint count and TP18 which further supports this theory. BDNF has a known role in pain processes in the central and peripheral nervous system where nociceptor derived BDNF is involved in inflammatory pain. [18] Release of BDNF from microglia in the central nervous system seems to be a part of mechanical hypersensitivity and allodynia. This supports the direct role of BDNF in pain perception. [40] In a study done on patients with knee osteoarthritis they showed a positive correlation between serum BDNF and pain, where increased BDNF in serum indicated more self-reported pain. [41] This contradicts with our results. However, we are looking at CD14+ cells and not BDNF in serum. Few studies have been done on BDNF produced by CD14+ cells and its involvement in pain processes. By our results one can speculate that the elevated pain perception in the patients with low BDNF production by CD14+ cells may depend mostly on a more active disease.

Several studies have shown that BDNF production in serum and plasma is decreased in patients with mood disorders. [22] In our study we only found a tendency of higher score on depression-VAS in the group with low production of BDNF in CD14+ cells. However, our study population is small and based on BDNF produced by CD14+ cells, why a conclusion on the role of BDNF by CD14+ cells and its relation to mood disorders cannot be done. When dividing our study population in subgroups of those who had anti-depressive treatment and those who did not, we found that there were 6 patients who had an anti-depressive treatment.

However, these patients were distributed evenly between the low and high BDNF group why there was no need to exclude them from the analysis between low and high BDNF. However, the optimal design to measure depression would be to look at a bigger study population where patients with and without anti-depressive treatment are analysed separately. In conclusion, our results indicate that BDNF has a role in pain in patients with RA, where a low BDNF production by CD14+ cells indicate elevated pain perception and that this may mostly depend on elevated disease activity and inflammation within this group. However, there is some evidence of that BDNF has a direct involvement in pain perception why further research regarding this topic is needed.

### ***BDNF, quality of life and functional ability***

In our study a low production of BDNF by CD14+ cells indicate a worse functional status by both HAQ and FIQ. In RA, HAQ is one the strongest predictors of long-term outcomes of RA. [9]. RA is a disease known for its negative impact on physical function and quality of life. [42] Thus, BDNF produced by CD14+ cells may have an important role in the physical function and quality of life in patients with RA.

The patients with a low production of BDNF by CD14+ cells scored significantly higher on the DASH questionnaire in all categories, indicating a worse function in the upper extremity including hand. In our study we found a significant difference in maximal hand strength on both the right and left hand. The low BDNF group had significantly lower hand strength. In a study made on hand strength in RA patients compared to individuals without disease, the RA patients had a lower hand grip. Grip strength also correlated to functional status measured by HAQ, where lower grip strength indicated worse functional status. Yet, they only found a weak correlation between grip strength and disease activity measured by DAS28. [43]

However, other studies have shown an association between disease activity (DAS28) and hand strength in patients with RA, where higher disease activity correlated with worse hand function and grip strength. [44] Thus, the observed difference in hand function may depend on more inflammation and higher disease activity in the low BDNF group. However, physical activity elevates peripheral levels of BDNF and thus there may be a reversed connection between hand status and BDNF. The group with low BDNF may not do as much physical activity and therefore have lower levels of BDNF. [45] Further, one aspect that could affect hand strength is difference in age in the groups. There was no significant difference in age between the groups. The possible difference in age, does not play a role in the difference in hand strength in our study population, as there was no significant correlation between age and maximal hand strength. However, a relation between age and hand strength is known where a higher age indicates lower hand strength. [46] Another aspect that may impact the results regarding hand strength would be disease duration. Studies has shown some correlation between longer disease duration and worse hand function in patients with RA. [47] There was no significant difference in disease duration between the low and high BDNF group. There was no correlation between disease duration and maximal hand strength. Thus, neither age nor disease duration was the main factor behind the results. In conclusion our results indicate that BDNF has a role in functional ability and quality of life in patients with RA. BDNF has a role in daily hand function and in hand strength indicating worse functional hand status in the patients with low BDNF production.

### ***Methodological considerations***

This study is a cross-sectional and explorative study. Explorative studies are used for investigating in the impact of different factors. There was no control group, instead this study focused on finding differences between patients with diagnosed RA. To be able to further answer the question about if inflammation affects the ability of monocytes to produce BDNF, a control group of healthy subjects would be preferred. The study population of 35 is small. However, the patients have several comparable characteristics which is a strength of this study. As mentioned before, a study population where all have an anti-rheumatic treatment and the same anti-rheumatic treatment would be more ideal. Another interesting perspective would be to look at newly diagnosed patients that have not received any immunomodulatory treatment to further understand the role of BDNF in RA. As well, in regard of measuring pain and depression, a population where patients with an anti-depressive treatment and those without would be compared separately may have been preferred. Since the study population is limited in this study, in the future studies including larger number of patients need to be done to validate the results that were presented. A large amount of data regarding the patients was available through several completed questionnaires, this strengthens the study. However, the questionnaires were filled in by the patients themselves without any influence from the staff involved in the study. There is a chance of misunderstanding and inaccurate information from the questionnaires. However, when calculating the different scores used to present results, one has tried to take these sources of error into account by using well established and standardized ways to calculate the scores of the questionnaires. Regarding the VAS, three answers were missing, why the study population differs in this section from the other sections, which is a source of error. There are very few studies done on the impact of BDNF produced by CD14+ cells and therefore it is harder to prove the importance of these findings by other studies. On

the other hand, a strength of this study is that it is one of the first that explores BDNF produced by CD14+ cells in RA, introducing a new area of research. However, to further understand the impact of BDNF produced by CD14+ cells, enhanced investigation in this topic is needed.

## **Conclusion and implications**

Our conclusion is that low production of BDNF by CD14+ cells has a strong connection to higher disease activity. Further, clinical inflammation seems to decrease the production of BDNF by CD14+ cells. Low production of BDNF also indicates more pain, reduced quality of life and worse functional status overall, and in the upper extremity including hand. In the future, the role of BDNF produced by CD14+ cells require more research to fully understand the clinical associations of BDNF in patients with rheumatoid arthritis.

## **Populärvetenskaplig sammanfattning**

### **Kliniska aspekter av brain-derived neurotrophic factor hos patienter med ledgångsreumatism.**

Kronisk ledgångsreumatism eller reumatoid artrit (RA) är en autoimmun sjukdom som innebär att det egna immunförsvaret attackerar normala vävnader som finns i kroppens leder. Denna attack leder till inflammation som i sin tur ger smärta, stelhet och funktionsnedsättning hos de drabbade patienterna. Det är ofta små leder, som lederna i handen, som drabbas. Brain-derived neurotrophic factor (BDNF) är ett protein som finns i stor grad i hjärnan och reglerar hjärnans funktion. BDNF finns även i celler som ingår i vårt immunförsvaret, dessa immunceller finns i vårt blod. Tidigare studier har visat att inflammation sänker nivåerna av BDNF i blodet. Underskott av BDNF kan således ha negativa effekter för patienter med RA.

I den här studien har vi analyserat samband mellan produktionen av BDNF i immunceller och sjukdomsaktivitet, smärta, fysisk aktivitet och handfunktion hos patienter med RA. 35 patienter som följs för sin RA på Reumatologkliniken, Sahlgrenska Universitetssjukhus, var med i studien. Dessa patienter svarade på enkäter, blev undersökta samt lämnade blodprov. Vi valde att studera immunceller som fanns i blodet för att studera deras förmåga att producera BDNF. Vi analyserade sedan patienterna i två grupper beroende på om de hade låg eller hög produktion av BDNF. Vi analyserade om det fanns någon länk mellan BDNF-produktion, sjukdomsaktivitet, livskvalitet, patientens fysiska aktivitet, smärta och handfunktion.

Vi upptäckte att de RA patienter som har låg BDNF-produktion har mer svullna och ömma leder och därmed högre sjukdomsaktivitet. Bland patienterna med lågt BDNF så hade 73% aktiv sjukdom medan enbart 25% av patienterna med hög BDNF-produktion hade aktiv sjukdom. Patienterna med lågt BDNF har även mer tecken på klinisk inflammation i och med fler svullna och ömma leder. Patienterna med lågt BDNF har mer smärta. Patienterna med lågt BDNF har även sämre handfunktion och lägre handstyrka än patienterna med hög BDNF-produktion. Att ha en funktionsnedsättning av handen och att ha mycket smärta påverkar livskvaliteten hos RA patienter. Patienterna med låg produktion av BDNF hade betydligt sämre resultat på Health Assessment Questionnaire (HAQ) som är ett frågeformulär som generellt används för bedömning av livskvalité.

Vår slutsats är således att hos RA patienter så finns det en koppling mellan låg produktion av BDNF i immunceller och hög sjukdomsaktivitet. Klinisk inflammation påverkar produktionen av BDNF hos immunceller. Låg produktion av BDNF tyder även på mer smärta, sämre livskvalitet och sämre handfunktion hos patienter med RA.

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## Abbreviations

RA: rheumatoid arthritis

BDNF: brain-derived neurotrophic factor

ELISA: enzyme linked immunosorbent assay

VAS: visual analog scale

ESR: erythrocyte sedimentation rate

CRP: c-reactive protein

TLR: toll-like receptors

HAQ: The health assessment questionnaire

DASH: The disabilities of the arm, shoulder and hand questionnaire

CNS: central nervous system

TrkB: tropomyosin receptor kinase B receptor

P75NTR: p75 neurotrophin receptor

AIA: adjuvant-induced arthritis

LPS: lipopolysaccharides

TP18: tender points 18

DAS28: disease activity score 28

FIQ: Fibromyalgia impact questionnaire

Hb: Haemoglobin

Anti-CCP: anti-cyclic citrullinated peptides

RF: rheumatoid factor (RF)

IFN- $\gamma$ : interferon-gamma

TNF-i: tumor necrosis factor inhibitor

DMARDs: disease-modifying antirheumatic drugs

CI: confidence interval

TNF- $\alpha$ : tumor necrosis factor- alfa

IL-6: interleukin 6