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Immunoglobulin A antibodies against phosphorylcholine in rheumatoid arthritis patients and its association with clinical outcomes

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disorder, primarily affecting the joints in wrist and hands. RA develops slowly and can cause physical disabilities which may lead to extra-articular manifestations, damaging other parts of the body such as heart and blood vessels. RA patients have an elevated chance of developing atherosclerosis or cardiovascular disease (CVD). This study investigated the role of natural IgA (immunoglobulin A) antibodies against phosphorylcholine (PC) in RA patients and its association with clinical outcomes.

IgA anti-PC levels were measured at baseline (day 1) and after 24 weeks by following an optimized indirect ELISA protocol in 682 patients with early rheumatoid arthritis. All patients followed the conventional treatment by receiving methotrexate. To this was included: Arm 1 (Active conventional treatment): prednisolone, or sulphasalazine, hydroxychloroquine. Arm 2 included certolizumab, Arm 3 included abatacept and Arm 4 included tocilizumab. Disease activity was determined by DAS28.

After 24 weeks, the mean value of DAS28 was significantly reduced in patients who reached remission (DAS28<2.6), p= 0.0001. Patients who achieved remission in week 24 did not show any significant difference with non-remission (DAS>2.6) patients in IgA anti-PC values at baseline. However, there may be differences in subgroups of patients with high IgA anti-PC at baseline, and also in the different treatment arms, which will be explored in further studies. The treatments with Arm 1, 2, 3 and its association with IgA anti-PC did not show any significant difference, except the treatment with Tocilizumab (Arm 4) and methotrexate which showed a significantly lower level of IgA anti-PC level after 24 weeks, p= 0.0143. One interesting possibility is that this treatment could increase the risk of CVD in the long run.

This present study offers new insights into the role of IgA anti-PC in the development of RA with different treatments. IgA anti-PC decreased during treatment, in the whole group. The results indicate that IgA anti-PC might be a novel biomarker for RA as the association between IgA anti-PC and disease activity in RA was elucidated to some extent. Some treatments decreased the levels of IgA anti-PC, which may have a negative effect on RA and may lead to a higher risk of developing atherosclerosis and CVD.

Populärvetenskaplig sammanfattning

Reumatoid artrit (RA) kännetecknas av inflammation i leder med smärta som främsta symptom och kan stegvis leda till andra sjukdomar som åderförkalkning och kardiovaskulära sjukdomar. Immunförsvarets funktion är att skydda mot infektioner genom att utlösa inflammation i kroppen. När immunsystemet angriper dess egna vävnader och celler resulterar det i en autoimmun sjukdom som RA. RA, åderförkalkning och kardiovaskulära sjukdomar är alla kroniska sjukdomar där intresse för att hitta olika botemedel ökar. För att hitta potentiella behandlingar mot reumatoid artrit är det av stor betydelse att förstå alla patologiska mekanismer bakom sjukdomen.

Idag finns inget botemedel mot reumatoid artrit, utan alla läkemedel har syftet att förebygga och lindra sjukdomen. Genom att använda Immunoglobulin A (IgA) antikroppar mot fosforylkolin (anti-PC) har det studerats om dess skyddande roll hos patienter med RA och dess association med kliniska resultat. Tidigare studier av professor Johan Frostegård har visat att en typ av antikroppar mot anti-PC, IgM, har skyddande egenskaper vid kronisk inflammation som åderförkalkning, kardiovaskulära sjukdomar och autoimmuna sjukdomen systemisk lupus erytematosus (SLE). Studier med IgA anti-PC är begränsade och har aldrig genomförts tidigare, därför har det valts att kolla dess effekt på patienter med tidig RA.

I denna studie användes serum från RA patienter där Enzyme linked immunosorbent assay (ELISA) utfördes för att mäta nivåer av IgA Anti-PC.

Patienterna i studien utvecklade lägre nivåer av antikroppar efter 24 veckor i jämförelse med baslinjen (dag 1), där skillnader fanns mellan de olika behandlingarna. Om IgA anti-PC minskar så kan risken för åderförkalkning och hjärtkärlsjukdom tänkas öka. Även om resultatet är lovande, återstår fortfarande fler djupgående studier av undergrupper och skillnader mellan de olika behandlingarna. Fortsatta studier i institutionen om mekanismer av behandling med IgA anti-PC kan förhoppningsvis bidra till bättre behandling vid reumatoid artrit.

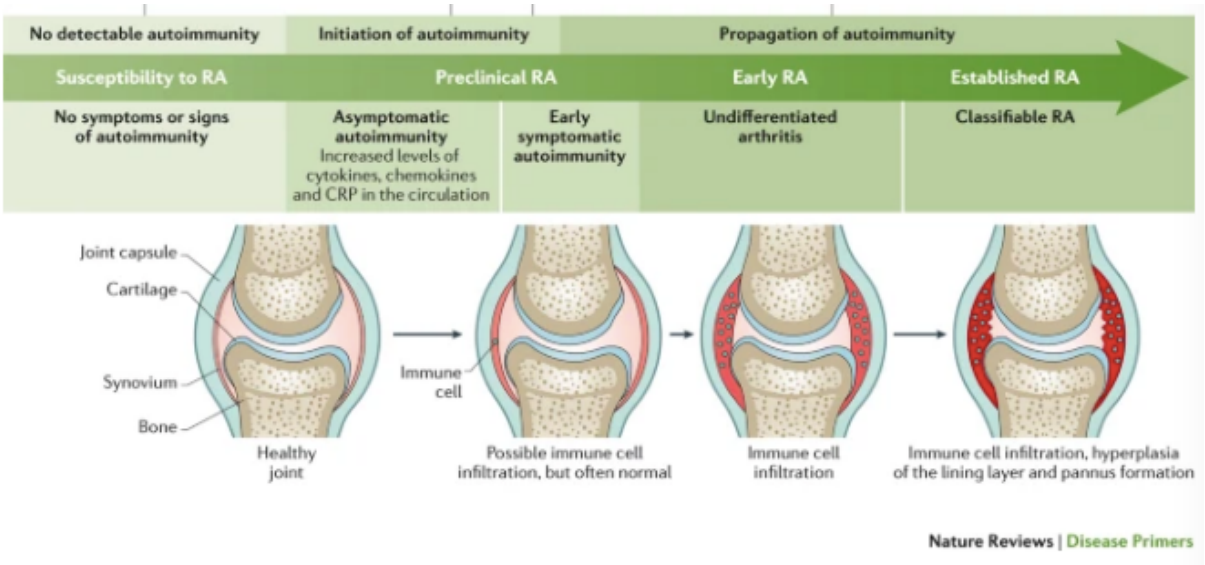
List of abbreviations

%	Percentage
ABA	Abatacept
ACPA	Anti-citrullinated protein antibodies
APC	Antigen presenting cell
B-cell	Lymphocyte developed in the bone marrow
BL	Baseline
BSA	Bovine serum albumin Degrees
°C	Degrees Celsius
COX	Cyclooxygenase
CRP	C-Reactive Protein
CV	Coefficient of variation
CVD	Cardiovascular disease
CZP	Certolizumab
DAMP	Damage associated molecular pattern
DAS28	Disease activity score in 28 joints
DC	Dendritic cell
DMARD	Disease modifying anti rheumatic disease
ECM	Extra cellular matrix
ELISA	Enzyme linked immunosorbent assay
GC	Glucocorticoids
HDL	High density lipoproteins
HLA	Human leukocyte antigen
H_2SO_4	Sulfuric acid
H2O	Water
IA	Intra-articular
IgA	Immunoglobulin A
IgG1	Immunoglobulin Gg1
IgM	Immunoglobulin M
IL-1	Interleukin 1
IL-6	Interleukin 6
IQR	Interquartile range
LDL	Low density lipoprotein
mL	Milliliters
N	Unit of Normality
nm	Nanometer
NSAID	Non-steroidal anti-inflammatory drugs
oxLDL	Oxidized low density lipoprotein
OD	Optical density
PAD	Peptidyl arginine deiminase
PAF	Platelet activating factor
PAMP	Pathogen associated molecular pattern
PBS	Phosphate buffered saline
PC	Phosphorylcholine
PG	Prostaglandin
PGA	Patient global assessment
RA	Rheumatoid arthritis
SAD	Systemic autoimmune disease
SMC	Smooth muscle cells
TCH	Triamcinolone hexacetonide
TCZ	Tocilizumab
T-cell	Lymphocyte developed in the thymus
Th1	T-helper cells
TLR	Toll like receptor
TNF-α	Tumour necrosis factor-α
TMB	3,3',5,5'-Tetramethylbenzidine
µg	Microgram
µL	Microliter
Vs.	*Versus*
W24	Week 24

1. Introduction

Rheumatoid arthritis (RA) is a combination of a musculoskeletal illness and systemic autoimmune disease (SAD), where the immune system mistakenly targets its own cells and tissues.[1] The most common parts affected include the smaller joints of hands. It can also occur in other parts where joints are found like wrists, elbows, shoulders, knees, hips and ankles.[2] The inflammatory disease has a higher prevalence in women than men and is estimated to affect 1% of the population globally. [3] Epidemiological studies indicated the prevalence rates to be higher in Northern Europe and North America, in comparison to Southern Europe. Currently, there is no cure for RA, however, with regular treatment, the symptoms and progression of the illness can be attenuated.[2] Thus, research into the pathogenesis of RA is of great importance to developing specific therapeutics and novel immunological targets.[4]

Figure 1:



Schematic illustration of the progression of rheumatoid arthritis. [5]

1.1. Immunological mechanism behind Rheumatoid Arthritis

The origin of RA is still ambiguous, however, a chronic inflammatory reaction is known to be involved.[6] As an autoimmune reaction takes place, inflammation occurs and clinical symptoms such as swelling, redness, pain, heat and loss of function starts occurring.[7] The active phase of

RA takes place when the synovial membrane in the joints starts to get inflamed. Autoantibodies react with self-antigens in the cartilage and the lining of the joint in the synovial membrane between the joints. An inflammatory response is induced resulting in the release of macrophages, mast cells and natural killer cells. These innate effector cells are recruited to Toll-like receptors (TLRs) where they bind, initiating an innate immune response.[8] The activated macrophages release pro-inflammatory cytokines, specifically tumour necrosis factor-alpha (TNF-α), interleukin 1 (IL-1) and interleukin 6 (IL-6). [9] [10] [11] Particularly, presence of TNF-α is one of the major pathogenic mediators of CVD.[11] Eventually, neutrophils and mast cells become a part of the process leading to chemokine production. Dendritic cells display the newly exposed self-antigen and activate T-cells in the joints or the local lymph node.[8] This triggers B-cells to start the antibody infiltration process by proliferation and produce antibodies that can identify self-proteins.[5] Fibroblasts, the cells in the joint lining thrive in the synovial fluid start growing and spreads to the cartilage surface. These cells secrete cartilage matrix enzymes causing an erosion in the tissue cartilage. Bone erosion, cartilage destruction and joint swelling contribute to the restricted movement in the joint and severe pain in RA. [1], [8], [10] Patients with RA have a higher morbidity and mortality rate associated with CVD due to inflammation in blood vessels leading to build-up of plaque, which is the fundamental cause of atherosclerosis. This is due to the extra-articular organ manifestations seen in the patients with acute illness.[11][12]

Several autoantibodies are recognized in RA resulting in autoimmunity, which eventually initiates the clinical symptoms in patients. Autoantibodies such as rheumatoid factor (RF) is of great importance for treating early RA.[4] One of the hallmarks in RA is known as citrullination and involves the occurrence of anti-citrullinated protein antibodies (ACPAs), which is one of the fundamental processes in recognising the autoimmune disorder.[13] ACPA production is triggered by a gene known as HLA-DR, specifically HLA-DR1 and HLA-DR4.[4,14] Citrullination is the conversion of arginine into citrulline through a post-translational modification with the enzyme, peptidyl arginine deiminase (PAD).[15] In RA patients, antigen-presenting cells (APCs) such as dendritic cells are found abundantly in the synovium that can recognize citrulline as a pathogen and initiate an inflammatory response.[16] Activation of the immune response initiates the production of the corresponding antibodies.[4] The inflammatory process is maintained in the synovium due to citrullinated proteins and enzymes, which leads to tissue destruction in the synovial membrane.[13] An abundant number of proliferating

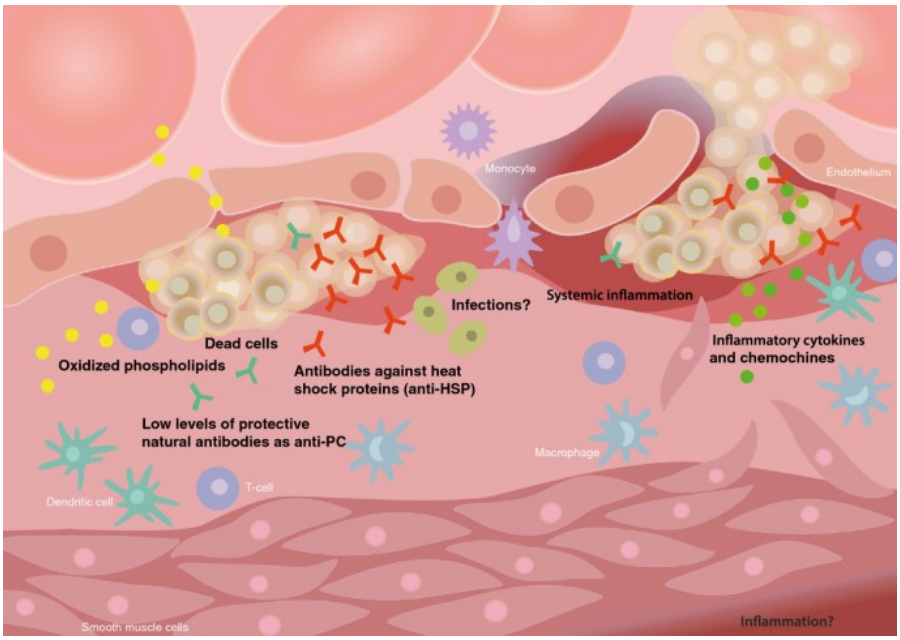
macrophages and lymphocytes are found in the synovial membrane, which stimulates B-cells to produce the corresponding antibodies.[4] Past findings have implied on citrullinated proteins to be a novel risk marker in the treatment of RA-related atherosclerosis and CVD.[17][18][19] Destruction in the synovial membrane and swollen joints also indicates having a higher level of the inflammatory marker, C-reactive protein (CRP). This was ultimately associated with cumulative carotid plaque leading to the destruction of endothelial and vascular damage, thereby the development of atherosclerosis and CVD. [20][21][22]

It has earlier been emphasized that RA-related autoimmunity arises at a mucosal site. Immunoglobulin A (IgA) is the predominant antibody isotype found in serum and the mucosal immune system. Additionally, IgA-ACPAs were displayed with high specificity for early RA patients and RA in preclinical patients. [6]

1.2. Atherosclerosis and CVD

CVD is one of the leading causes of death globally and involves diseases such as ischemic heart disease, stroke, congestive heart failure, rheumatic heart disease and coronary artery disease.[23] Atherosclerosis is the major underlying cause of developing CVD and has been associated with RA.[24]

Figure 2:



Schematic illustration of the build-up of plaque in atherosclerosis with the immunological mediators involved. [24]

The increased risk of CVD in RA occurs due to factors such as dyslipidaemia, smoking, hypertension and diabetes. There are many underlying causes of the inflammatory disease, atherosclerosis. One is the presence of dyslipidaemia in RA with high triglycerides and low high-density lipoprotein (HDL) levels. The fact that modified level of lipids such as Low-density lipoprotein (LDL) is a determinant factor for developing atherosclerosis has also been recognized in RA. [17] Finding from the past have demonstrated the mechanism of LDL undergoing a modification through oxidation resulting in the pro-atherogenic response to increasing.[25]

One theory about oxidized low-density lipoproteins (oxLDL) playing a significant role in the formation of atherosclerosis was suggested by Daniel Steinberg in 1980s. [22,26] Numerous dead cells and oxLDL start to build up on proteoglycans of the extracellular matrices (ECM) on endothelium cells and enters the intima of the arteries with age. [25,27] The presence of antigens such as accumulated oxLDL initiates a cascade reaction where an inflammatory response is activated and cytokines such as monocytes are released. [28] Activation of T-cells, mostly T-helper 1 (Th1) cell response is involved in the formation of lesion and plaque occurrence in the process of atherogenesis. The build-up of fatty deposits as LDL mainly takes place in the intima of arteries.[30] Monocytes are drawn to the damaged site where they get stimulated from oxLDL and differentiate into macrophages.[31] Macrophages take up oxLDL by scavenger receptors which leads to the formation of foam cells.[25][30] An increased amount of foam cells are found in the intima, where the dead foam cells turn into plaque.[30][24] Smooth muscle cells (SMCs) packed with cholesterol are found in the damaged site on the endothelium in the intima. SMCs are transported from the tunica media to the intima to proliferate and attach to the surface of the plaque, building up a calcification. Collagen and elastin produced by SMCs in the intima functions as a fibrous cap layering over the plaque. The aggregation of macrophages, SMCs and T-cells together in the arterial wall initiates the development of lesion and atherogenesis. [32,33] The immunological reaction may lead to narrowing of the blood vessel restricting the blood flow, thus inducing CVD. The theory about build-up of such lipids leading to an increased expression of proinflammatory cytokines is being discussed as a key hypothesis in modern research.[24]

1.3. Phosphorylcholine

Phosphorylcholine (PC) is an epitope with antigenic properties. Earlier reports suggest about PC being exposed on oxLDL in plaque and on apoptotic cells. It is also found on bacteria such as *Streptococcus pneumoniae*, parasites and microorganisms.[25] Thereby, PC functions both as a damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP).[24][34] PC being a significant component of a phospholipid, which is platelet-activating factor (PAF) contributes to platelet aggregation and is a mediator of inflammation.[22][35] The presence of PC is shown to contribute to the build-up of ox-LDL.[24] The surface of membranes in lipoproteins is exposed to PC, where the immune defence system recognizes this during apoptosis or LDL oxidation. The binding of PC to TLR (toll-like receptor) initiates the production of proinflammatory cytokines like TNF, IL-12 and some chemokines.[25] Prior research demonstrated antibodies against PC (anti-PC) to reduce the risk of developing cardiovascular diseases in humans and thus have atheroprotective and anti-inflammatory properties.[37] These results have led to a hypothesis about anti-PC being of great significance in the treatment of atherosclerosis.[25,36]

RA and CVD have some shared risk factors such as obesity, smoking, hypertension and dyslipidaemia lipid levels. There are both environmental and genetic factors involved in developing these conditions. However, age and gender also have an impact on the rupture of Plaque.[21] Atherosclerosis involves a mechanism that may lead to atherothrombosis. This happens when there is a destruction in the plaque due to cytokines and chemokines on the fibrous cap. The destruction of plaque results in exposure of prothrombotic material to the coagulation system which induces CVD and reduces blood flow. [24]

1.4 Treatments of Rheumatoid arthritis

Treating early RA is important to control the disease aggravation, where damage joints and other organs are included.[37] Nowadays, there are several therapeutic options available for RA. Although different pharmacological therapies are available to treat RA, there is no cure for this disease.[39] As there is a high possibility of developing atherosclerosis in RA, other risk factors such as dyslipidaemia, hypertension, smoking and obesity should always be taken into consideration. [40] First-line agents for acute pain relief and swelling include Non-steroidal anti-

inflammatory drugs (NSAIDs). These drugs work by inhibiting the production of prostaglandins (PGs) by inhibiting the enzyme cyclooxygenase (COX).[39] Other treatments include glucocorticoids (GCs), which are taken by intraarticular injections for acute relief.[40]

There are also long-run treatments available, known as Disease-Modifying Anti-Rheumatic Drugs (DMARDs) and conventional antirheumatic drugs e.g. Methotrexate and Sulphasalazine, which are recommended for early treatment.[41,42] The action of mechanism of Methotrexate is by inhibiting the activation of cell lineages in T cells, macrophages, endothelial cells and fibroblast-like synoviocytes.[42] Hydroxychloroquine is one of the DMARDs and is involved in the inhibition of various innate and adaptive immune processes. The mechanism of action is by inhibition of TLR signalling resulting in less production of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF by B cells and T cells.[44]

If no reduction is seen in the disease activity with the above mentioned treatments, biological DMARDs can be added.[45] The frequently used treatment in early treatment includes anti-TNF drug such as Certolizumab, which blocks the specific proinflammatory cytokine, TNF-α that plays a major role in the development of RA.[45,46] Thus, the underlying causes are treated rather than only treating the symptoms. Studies have in the past indicated anti-TNF drugs to have a positive effect on CVD in humans.[47] Tocilizumab (TCZ) is also included in biological DMARDs that target IL-6 receptors reducing the inflammation.[43] Abatacept is another agent, which targets the activation of T-cells and is a T-cell co-stimulation blocker leading to a decrease in the expression of CD28 in CD4+ cells in RA patients. A reduction of biomarkers among others CRP, IL-2, IL-6 and TNF-α is seen, which is significant in RA patients.[48] However, more studies are required to decide if one or more biologics could be more effective or safer and better suited to various types of patients with RA.

1.5. Disease activity of Rheumatoid arthritis

The activity of RA is measured by a scoring system known as disease activity score in 28 Joints (DAS28). This system makes it easier to evaluate the efficacy of the ongoing treatment. Several parameters are taken into consideration when evaluating the activity such as tender joints, patient global assessment (PGA), swollen joints and CRP value from the laboratory. Having a DAS28 value of ≤ 2.6 indicates the patient is in the remission phase. Whereas, values 3.2 <

DAS28 ≤ 5.1 and ≤ 3.2 indicates having a moderate and low disease activity of RA. A value of DAS28 ≥ 5.1 indicates a high diseaseactivity of RA. Based on the value, the right treatment can be given to the patient.[49]

A recent study reported the significant role of natural immunoglobulin M (IgM) antibodies against PC in the chronic rheumatic disease, systemic lupus erythematosus (SLE). The findings from the study indicated to have protective characteristics for developing atherosclerotic plaques. The findings of having low levels of anti-PC were linked to a much lower and almost no response to biological drugs in RA candidates, particularly a negative association.[50] Low levels of anti-PC were anticipated to increase the possibility of developing CVD and intensify the progression of inflammation.[17] In 2020, it was reported by Thiagarajan *et al*. that IgM anti-PC is a protection marker in CVD development in a cohort group of 60-years old.[50] Although IgA anti-PC has not been acknowledged in association with RA and atherosclerosis, another subtype of IgG, namely IgG1 has been associated with progression in atherosclerosis.[51]

1.6. Aim of this study

The underlying immunological mechanisms of RA and atherosclerosis are well known together with the role immunity plays. A strong association between RA and atherosclerosis have been reported in many papers. The fact that a great number of studies has been done on IgM has led immunoglobulin A (IgA) anti-PC to be out of focus due to which a deeper study has not been taken into consideration. The objective of this thesis was to study the effect of IgA anti- PC in patients with early RA from the NORD-STAR cohort following four different treatments, active conventional therapy and three other biological treatments to see the association with the clinical outcomes. Potential immunomodulatory therapies can further be developed using the antibody to target the root causes of the inflammation and treat patients with early RA, thereby atherosclerosis and CVD.

1. Materials and Method

2.1. The Cohort of Nordic Rheumatic Diseases Strategy Trials (NORD-STAR)

In 2011, adults with early RA started to enter an international trial from the Nordic countries; Sweden, Norway, Denmark and Finland as well as Island and Netherlands. In this study, a total of 682 patients with RA were recruited from the Nordic rheumatic Diseases Strategy Trials (NORD-STAR) till 2018, where their serum samples were collected from Karolinska Institute and stored at -80 °C. The cohort aims to see the numbers of patients that can reach the remission phase with a normal treatment in comparison to three different biological treatments. All patients followed the conventional treatment by receiving methotrexate (25mg/week).

To this was included:
Arm 1 (Active conventional treatment): prednisolone (decreased from 20mg/d to 5 mg/d in 9 weeks) or sulphasalazine (2 g/day), hydroxychloroquine (35 mg/kg/week) together with obligatory intra-articular (IA) triamcinolone hexacetonide (TCH) in swollen joints ≤ 4 (≤80 mg/visit up to week 20).
Arm 2 (CZP) included certolizumab 200 mg every other week (EOW) subcutaneously (SC) (400 mg at 0, 2 and 4 weeks)
Arm 3 (ABA) included abatacept 125 mg/week subcutaneously
Arm 4 (TCZ) included tocilizumab 8 mg/kg/4weeks IV or 162 mg/week subcutaneously.
The patients were followed till week 24 and their disease activity was calculated by DAS28 (0-9.4).

2.2. Study population

The population in the NORD-STAR trial were amongst early rheumatoid arthritis patients according to the American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) 2010 classification criteria. RA patients from age 18 years were recruited who had symptoms for less than 24 months. The study can be described as a multicentred, randomized and open-label double-blinded trial in patients with early RA in phase 4. Other inclusion criteria included moderate to severe disease activity with DAS28 greater than

3.2, at least two (of 66) swollen and minimum two (of 68) tender joints, and RF or ACPA, or CRP at least 10 mg/L. Exclusion criteria included past treatment with DMARDs.

2.3. Ethical considerations

The study was approved by the ethics committee at Karolinska Institute, Stockholm, Sweden. The study was accomplished in accordance to the Helsinki declaration.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Levels of IgA anti-PC were measured following an indirect ELISA protocol. Serum samples of 682 patients from the NORD-STAR cohort were available for antibody level determination. Serum from the patients was collected at baseline (day 1) and after 24 weeks follow up and kept at -80 °C until the day of analysis. The experiment was conducted by coating Nunc Immuno microwell plates (Thermo Labsystems, Franklin, MA, USA) with 10 μg/ml concentration of PC -Bovine Serum Albumin (PC-BSA) at 100 μl/well. The plates were kept at 4 °C overnight for incubation. On day two of the experiment, the plates were washed with 300 μl wash buffer four times (Biotek plate washer ELx 50) followed by blocking the plates with 2% BSA- Phosphate Buffered Saline (BSA-PBS) 200 μl/well for an hour. The samples, internal controls, and standards were prepared by diluting 1:200 with 0,2% BSA at 100 μl/well. Pooled serum was used as standard control from Sigma for all plates. Plates were kept for two hours at room temperature and were washed with 300 μl was buffer four times. This was followed by adding Biotin-conjugated rabbit antihuman IgA diluted 1:15000 with 1% BSA-PBS at 100 μl/well. After two hours of incubation, horseradish peroxidase-conjugated streptavidin (Thermo Scientific, Roskilde, Denmark) was diluted 1:5000 in 0,2% BSA-PBS. Plates were washed once again after exactly 20 minutes. The color was observable after adding 100ul per well of horseradish peroxidase substrate, 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma Aldrich, St. Louis, MO, USA), and incubating the plate in darkness for 10 minutes at room temperature. The last step was to add 50 μl of the stop solution 1 N H_2SO_4 in all of the wells to stop the reaction. Lastly, the plates were analyzed in an absorbance reader (BioTek 800TS) at 450 and 630 nm. The samples were in an assay measured in duplicates. The optical density (OD) was measured at 450 nm and 630 nm and between the duplicates, the coefficient

of variation (CV) was below 15% for IgA. The activity level of IgA was measured in Microsoft Excel.

The unit values for each sample was measured by using the equation:
Unit Value= (Delta (Sample)) / (Delta (Standard)) X 100
Delta value was measured by subtracting blanked OD at 630 nm from the blanked OD at 450 nm. The Delta value of the respective sample was later divided by the Delta value of the standard to attain a relative Unit value of the abundance of antibodies in this sample.

All experiments in this study were successfully performed by Amna Syed.

2.5. Statistical analysis

All experimental statistical analysis was presented using the software Graphpad Prism 8, Prism 9 and Microsoft Excel. The P-value in the analysis was an observation of the association between IgA anti-PC and DAS28 levels in the disease activity at baseline and week 24. Data from the analysis were expressed in Whiskers box-plot as median and interquartile range (IQR). The level of statistical significance was fixed to $p < 0.05$. Results are presented in box-plot with minimum to maximum value.

2. Results

The association between the presence of IgA anti-PC and disease activity of RA was studied in early RA patients from the NORD-STAR cohort through ELISA. The levels of IgA anti-PC were analysed from the patient's serum by following an in-house ELISA protocol. Statistical analysis was proceeded through paired and unpaired t-test depending on the number of people and statistical significance is specified with a p-value.

3.1. Anti-PC IgA levels associated with clinical outcomes

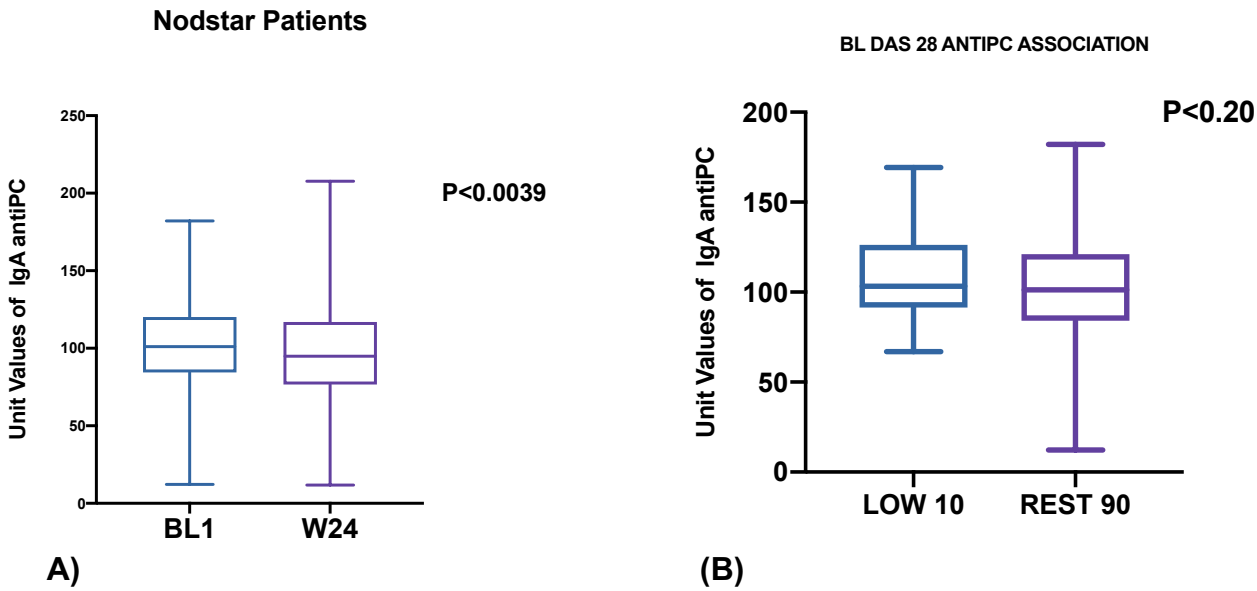


Figure 1. ***(A)*** *Levels of IgA anti-PC in baseline (BL) (n= 341) and week 24 (W24) (n=341).* ***(B)*** *Association between IgA anti-PC levels and disease activity in baseline of DAS28 in lowest 10th percentile (n=33) and rest 90th percentile (n=313).*

The figure is divided into two box-plots; level of IgA anti-PC in the serum from baseline (BL) and from the follow up after 24 weeks. The IgA anti-PC value in patients from week 24 is decreased significantly (median [interquartile range]: 101,1 [84,38-120,2] versus 94,84 [76,66-116,9]; P= 0.0039); **Figure 1A**. The patient's DAS28 in baseline in the lowest 10th percentile and the rest 90th percentile (median [interquartile range]: 103,3 [91,36-126,3 versus 101,3 [84,16-121,0]; P= 0.20); **Figure 1B**. The patients in the baseline (BL) did not have any significant values of IgA anti-PC level associated with DAS28 in the lowest 10th percentile and in the rest 90th percentile.

3.2 Association between IgA anti-PC levels and disease activity

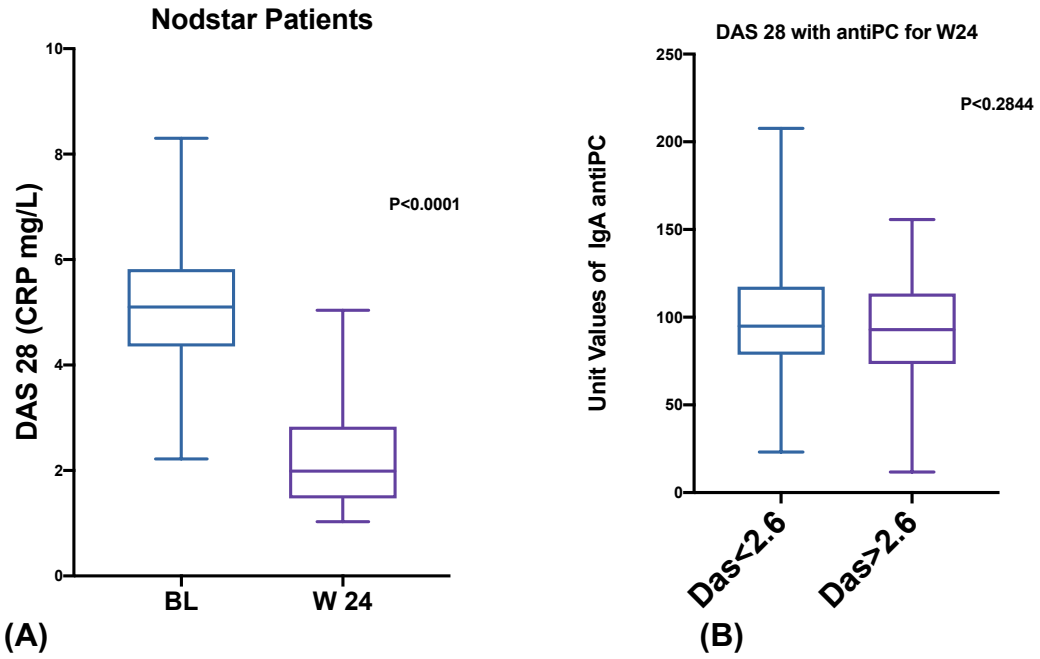


Figure 2. *(A) Disease activity score in 28 joints (DAS28) (CRP mg/L) in baseline (n=340) and after follow-up period in week 24 (n=340).* ***(B)*** *Levels of IgA anti-PC after follow-up at week 24 from patients with remission (DAS <2.6) (n=239) and non-remission (DAS >2.6) (n=100).*

In baseline, a statistically significance was seen with higher DAS28 (CRP mg/L) level in comparison to DAS28 value in the follow up period in week 24 (median [interquartile range]: 5,1 [4,353-5,820] versus 1,99 [1,470-2,830]; P= 0.0001); **Figure 2A.** DAS28 values after 24 weeks follow up were declined, seen in **Figure 2A.**
The disease activity in week 24 did not show any significant difference of IgA anti-PC levels in DAS<2.6 (remission) and DAS> 2.6 (non-remission) (median [interquartile range]: 94,94 [78,62-117,4] versus 92,88 [73,30-113,5]; P= 0.2844); **Figure 2B**.

3.3 DAS28 association with IgA anti-PC in baseline and week 24

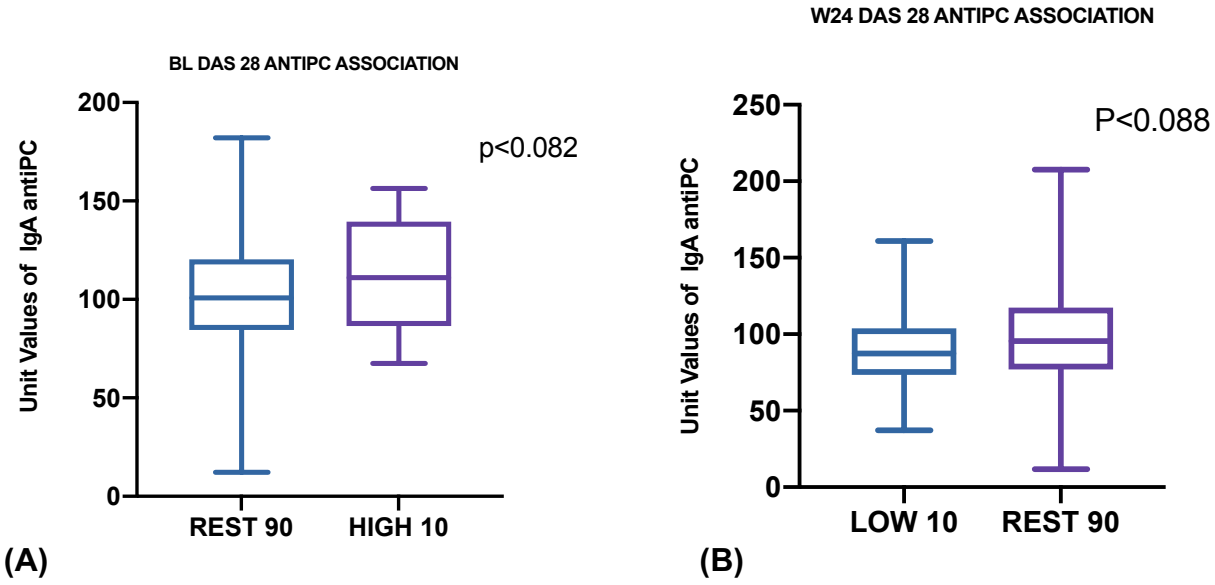


Figure 3. *(**A**) Levels of IgA anti-PC in baseline of DAS28 in rest 90th percentile (n=315) and highest 10th percentile (n=31) of the disease activity in baseline. (**B**) Levels of IgA anti-PC in patients with DAS28 in lowest 10th percentile (n=33) and rest 90th percentile (n=310) in week 24.*

The disease activity in baseline did not show any significant levels of IgA anti-PC in the rest 90th percentile and highest 10th percentile (median [interquartile range]: 100,8 [84,50-120,4] versus 111,0 [86,49-139,5]; P= 0.082); **Figure 3A**.

There was no significant difference in the disease activity after 24 weeks in IgA anti-PC levels in the lowest 10^{h} percentile and in the rest 90th percentile (median [interquartile range]: 87,45 [73,42-103,9] versus 95,47 [76,97-117,4]; P= 0.0888); **Figure 3B**.

3.4 Association of DAS28 with IgA anti-PC in week 24

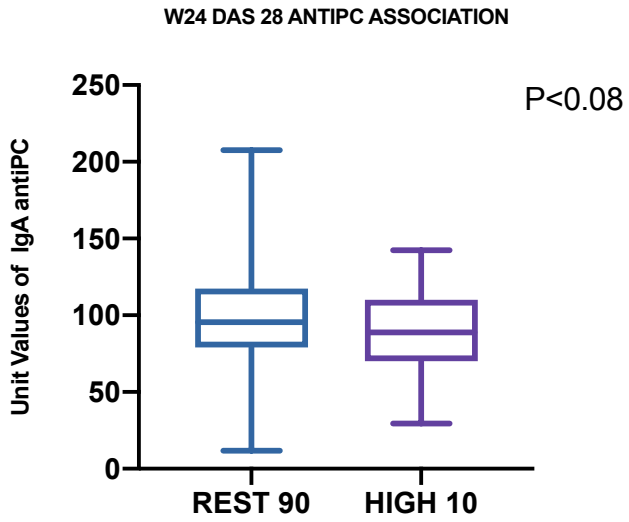
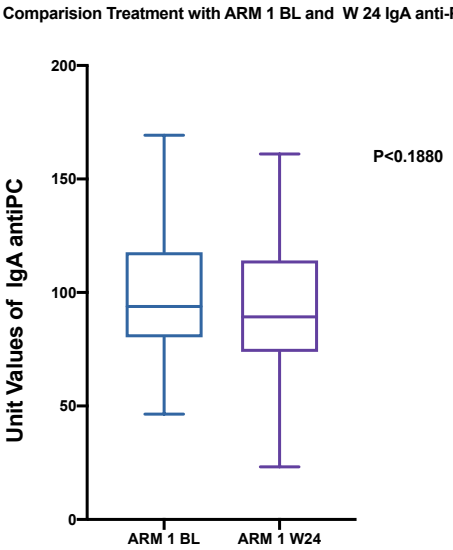


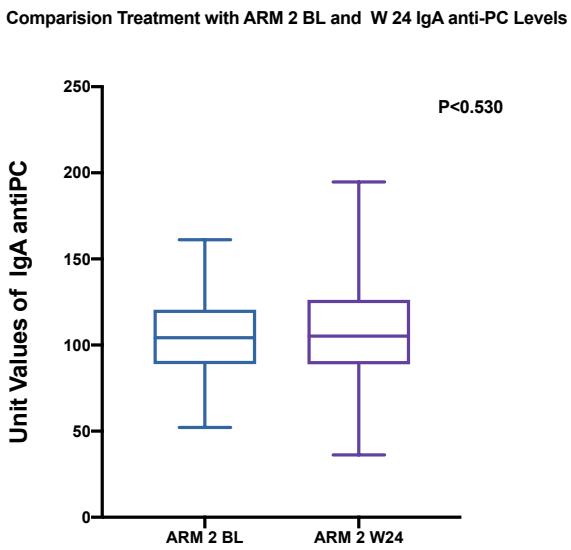
Figure 4. *Levels of IgA Anti-PC in week 24 (W24) in the rest 90th percentile of DAS28 (n=313) and remaining high 10th percentile(n=32) of DAS28 value.*

The disease activity in week 24 did not achieve a significant result in the 90th percentile and highest 10th percentile (median [interquartile range]: 95,42 [79,03-117,3] versus 88,82 [70,05-110,2]; P= 0.08); **Figure 4**.

3.5. Comparison of the treatments with IgA Anti-PC in BL and W24



(A)



(B)

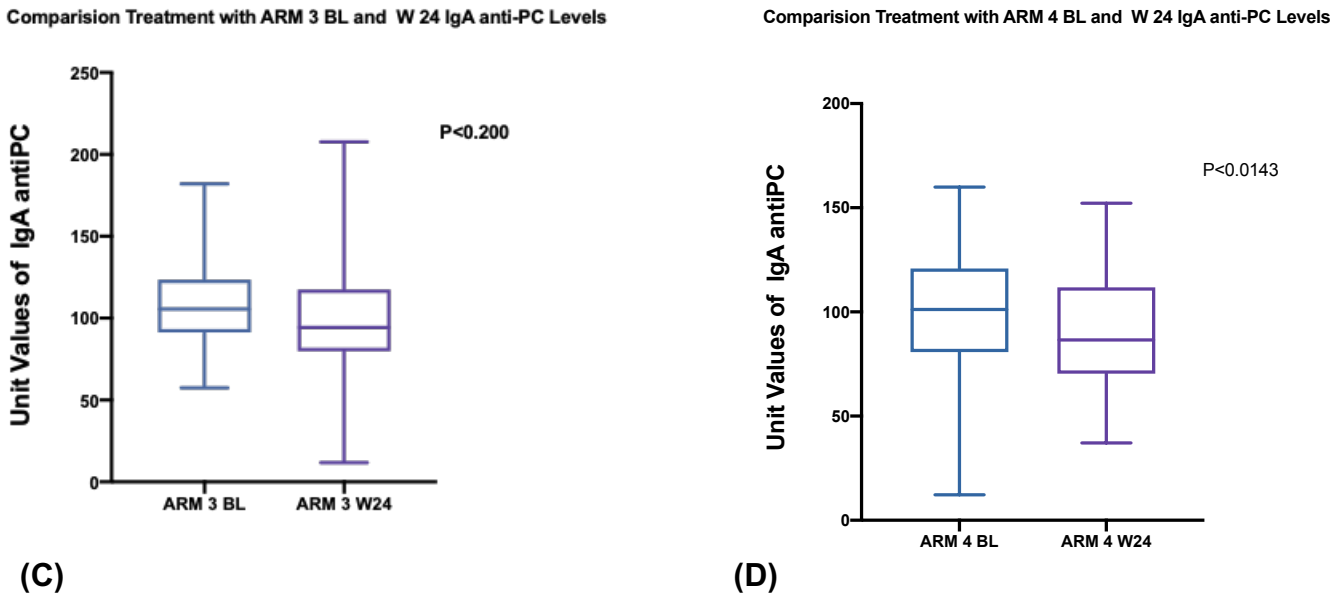


Figure 5. *Changes in IgA anti-PC levels in baseline (BL) and after the follow-up in week 24 (W24) according to the treatment;* ***(A)*** *ARM1: prednisolone or sulphasalazine, hydroxychloroquine together with obligatory intra-articular (IA) triamcinolone hexacetonide (TCH). BL (n=84), W24 (n=82).* ***(B)*** *ARM 2: Certolizumab (CZP)+Methotrexate. BL (n=87), W24 (n=88).* ***(C)*** *ARM3: abatacept (ABA) + Methotrexate. BL (n=92), W24 (n=91).* ***D)*** *ARM 4: Tocilizumab (TCZ)+ Methotrexate. BL (n=84), W24 (n=82)*

The figures are showing levels of IgA anti-PC in the serum from baseline (BL) and from the follow up after 24 weeks. The patients following treatment with ARM 1 did not show any significant difference in anti-PC levels from baseline to the follow up period in week 24 (median [interquartile range]: 93,82 [80,29-117,7] versus 89,23 [73,85-114,1]; P= 0.1880); **Figure 5A**. Levels of IgA anti-PC in the patients following treatment with ARM 2 at baseline and in the follow up period in week 24 did not show any statistically significant difference (median [interquartile range]: 104,3 [88,90-120,5] versus 105,2 [88,87-126,2]; P= 0.530); **Figure 5B.** The patients following treatment with ARM 3 did not show any statistically significant difference in anti-PC levels from baseline to the follow up period in week 24 (median [interquartile range]: 105,5 [91,22-123,5] versus 94,27 [79,80-117,5]; P= 0.200); **Figure 5C**. Levels of IgA anti-PC in the patients following treatment with ARM 4 in baseline and after 24 weeks follow up did show a statistically significant difference (median [interquartile range]: 101,2 [80,73-120,8] versus 86,56 [70,33-111,8]; P= 0.0143); **Figure 5D.**

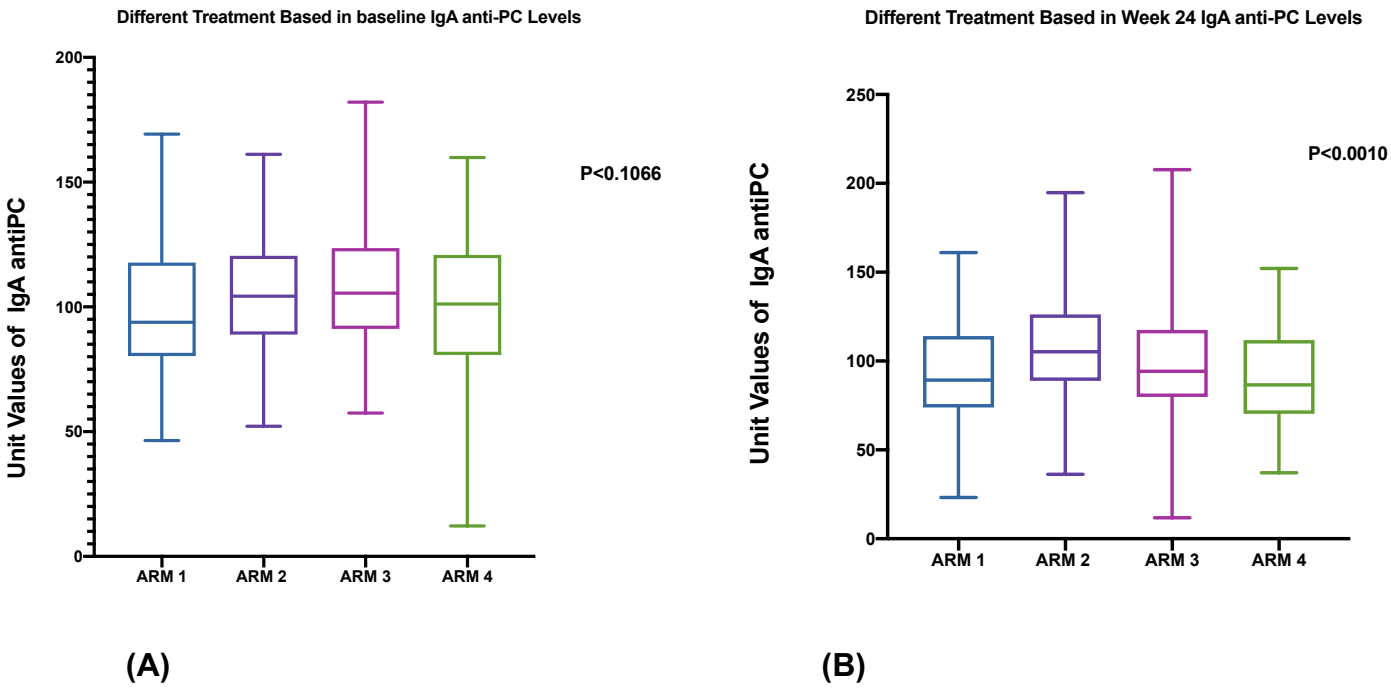


Figure 6. (A) *Levels of IgA anti-PC in baseline according to the different treatments; Arm 1 (n= 84), Arm 2 (n= 87), Arm 3 (n=92), Arm 4 (n= 84).* ***(B)*** *Levels of IgA anti-PC after follow-up period in week 24 according to the different treatments; Arm 1 (n=82), Arm 2 (n= 88), Arm 3 (n=91), Arm 4 (n= 82).*

The figure is divided in four box-plots showing levels of IgA anti-PC in the serum from baseline and from the follow up after 24 weeks. IgA anti-PC levels did not show any statistically significant difference in baseline (median [interquartile range]: (93,82[80,29-117,7] versus 104,3[88,90-120,5] versus 105,5[91,22-123,5] versus 101,2 [80,73-120,8]; P= 0.1066); **Figure. 6A.** The levels of IgA anti-PC in week 24 showed a statistically significant result (median [interquartile range]: 89,23 [73,85-114,1] versus 105,2[88,87-126,2] versus 94,27[79,80-117,5] versus 86,56[70,33-111,8]; P= 0.0010); **Figure 6B.**

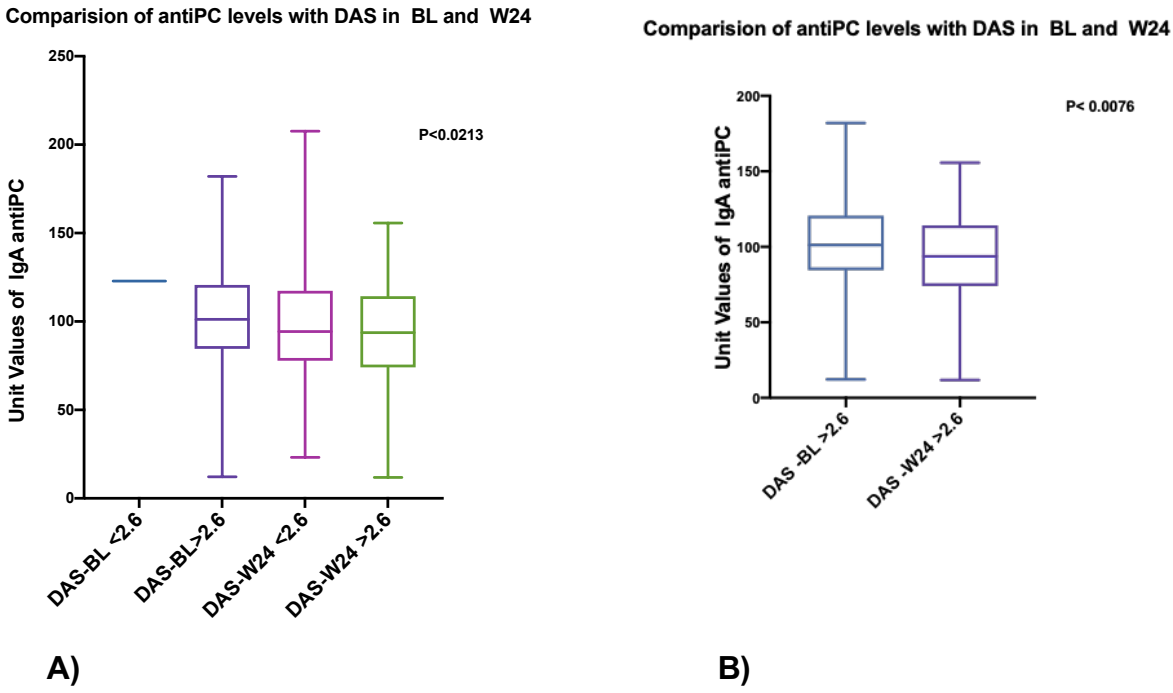


Figure 7. ***(A)*** *Levels of IgA Anti-PC in comparison with DAS28 (<2.6 and >2.6) in baseline and in week 24. DAS-BL<2.6 (n=1), DAS-BL>2.6 (n=345), DAS-W24<2.6(n=241), DAS-W24>2.6(n=104)* ***(B)*** *IgA anti-PC level for patients in remission (DAS>2.6) in baseline (n=345) and in week 24(n=104).*

Levels of IgA anti-PC in patients from baseline with DAS28<2.6 and DAS28>2.6 and in patients from week 24 DAS28<2.6 and DAS28>2.6 are shown in the figure with a statistically significant difference (median [interquartile range]: (122,9[122,9-122,9] versus 101,3[84,51-120,7] versus 94,34[77,83-117,3] versus 93,74[74,07-114,3]; P= 0.0213); **Figure. 7A.** Levels of IgA anti-PC in patients from baseline with DAS28>2.6 and from week 24 in patients with DAS28>2.6 with a statistically significant difference are seen (median [interquartile range]: (101,3[84,51-120,7] versus 93,74[74,07-114,3]; P= 0,0076); **Figure 7B.**

4. Discussion

This prospective observational study has established an important discovery of IgA anti-PC in RA patients, mainly that the levels of IgA anti-PC decreases after the follow-up period in week 24 as compared to the level in the baseline. There are interesting differences between the arms of treatment where there was no significant decrease (mainly non-significant decrease) in three arms, while a clear and significant decrease in one. There was no significant association between baseline IgA anti-PC levels and outcome after 24 weeks, as determined by the disease activity measure DAS28. It is still possible that some of the subgroup or treatment arm had an association with protection by IgA anti-PC level. This analysis has not been done yet and a longer follow up period with more patients and data may elucidate the association between treatments and IgA anti-PC with the outcome.

The inflammatory marker, CRP used for measuring the disease activity of RA known as DAS28 was also analysed. In the baseline, a higher value of DAS28 (>5.1) indicated the patients having a high disease activity of RA. In week 24, the DAS28 value decreased below 2.6 which corresponds to the patients being in the remission phase. This finding also implies on many patients responded to the treatment as remission was observed and has recently been reported. [60] Which specific treatment is primarily resulting in remission was not investigated in this study. Also, high levels of IgA anti-PC in baseline for remission were not examined in this study, which will be proceeded in future studies, and it is still possible that high levels of IgA anti-PC at baseline are associated with protection at follow up in week 24.

All four conditions of DAS28 in baseline and week 24 had a statistically significant difference in the median value of IgA anti-PC. There is not a big difference in the median value in DAS28<2.6 and DAS28>2.6 from week 24. IgA anti-PC levels were higher in DAS28>2.6 in the baseline as compared to DAS28>2.6 in week 24. The levels of IgA anti-PC being reduced

in week 24 in patients with DAS28>2.6 may imply on the subgroups included in this condition having a higher possibility of developing atherosclerosis and CVD.

A previous finding focused on IgM anti-PC, where high levels played a significant role in chronic inflammatory diseases. The association of high IgM anti-PC levels in systemic autoimmune diseases such as SLE and RA have in the past been studied with a protective potential and significant negative associations.[50] The study on RA-patients showed low levels of anti-PC being associated with non-response to biological drugs.[52] A study suggested that anti-PC IgM could contribute to positive effects in atherogenesis and chronic inflammation with a negative association in the development of atherosclerosis. Several past studies have reported low levels of anti-PC increasing the risk of atherosclerosis and CVD development.[22,24,47,53] Therefore, the role of IgA was investigated to understand the part it plays in RA.

It is well known that RA patients have a higher mortality rate and increased risk of CVD in comparison to people without RA.[54] Gradually, extra-articular manifestations of RA can arise affecting other organs including the heart.[12] In patients diagnosed with RA, there are high risk of developing CVD which is likely to be associated with risk factors such as oxidation contributing to PC sensitivity and low anti-PC levels. Anti-PC is a contributing factor in decreasing the disease activity of RA and thereby CVD. A mechanism of anti-PC being protective is by inhibiting the uptake of Ox-LDL into the macrophages found in foam cells. It may also prevent apoptosis caused by a significant component of OxLDL. Furthermore, anti-PC inhibits the inflammatory phospholipid effects due to its anti-inflammatory properties. These underlying mechanisms may be involved in chronic inflammatory diseases; RA and CVD.[50] In early reaction to fatal infections with PC- exposing *streptococcus pneumoniae*, anti-PC natural antibodies are known to be protective in mice. It is thus shown that PC can play both protective and harmful roles in immune reactions. Findings have shown a negative association between anti-PC and atherosclerosis.[55]

Centred on the results outlined above, a novel theory has been suggested that low levels of anti-PC indicate an immune defect associated with an elevated risk of chronic inflammatory disorders, such as atherosclerosis. Moreover, through its anti-inflammatory properties and also likely through its anti-apoptotic role, low anti-PC can promote disease development and disease outbreak in autoinflammatory conditions.[56]

PC has been present for a long time and the part it has in immunological reactions have previously been stated, however little is known about IgA anti-PC in autoimmune disease development in humans.[57]

All patients were following one out of four arms together with the DMARD, Methotrexate. Use of any DMARDs or specifically methotrexate have earlier been associated with a better outcome in CVD. The mechanism of action behind is believed to be effective due to a long-lasting control of systemic inflammation.[56] Observations in this study may have influence of methotrexate. However, more studies are necessary to understand the influence of each drug on IgA anti-PC levels in RA patients.

As discussed earlier, the levels of IgA anti-PC are reduced slightly after 24 weeks. Further experiments would be required to find the arm that may have an impact on IgA anti-PC levels. IgA anti-PC values did not significantly differ in RA-patients administered with active conventional treatment with DMARDs (Arm 1) after 24 weeks in comparison to baseline. Therefore, this could imply that anti-PC IgA might not be protective for RA-patients receiving active conventional treatment with methotrexate. A certain number of patients following arm 1 were administered glucocorticoid (GC), prednisolone. In a past study from 2007 by Hafström *et al*. Prednisolone usage was shown to be amongst a risk factor for death in RA. For a longer usage of prednisolone, it was reported to improve the development of RA by inhibiting inflammation in the arterial wall, which was later supported by clinical studies in rabbits.[58]

The RA-patients following arm 2, treatment with certolizumab and methotrexate did not show any significant difference in the median for the baseline and after week 24. The mechanism of certolizumab, which acts as a TNF-α inhibitor together with anti-PC is not fully known. One hypothesis about the association between anti TNF-α and anti-PC is the drug having a strong inhibitory effect on anti-PC producing B-cells. There may be a possibility of IgA not having any association with RA and thereby not increasing the levels of IgA anti-PC. Treatment with anti TNF-α may lead to a reduction in coagulation and modify CVD risk associated with RA.[11]

Patients following arm 3 received the biological treatment, abatacept together with methotrexate showed no significant difference in week 24, but a slight reduction was observed in week 24. This finding with patients being administered with abatacept may indicate the

treatment not being of any significance in the long-run. Although little is known about the potential underlying mechanism of anti-PC together with abatacept, it may be something to look into in future studies.

A significant difference was observed in the median value for RA patients following arm 4 (Tocilizumab) after 24 weeks. Tocilizumab is an immunosuppressive drug which acts through inhibiting IL-6. IgA anti-PC levels were significantly decreased after 24 weeks in arm 4 which may indicate on Tocilizumab and methotrexate together with IgA Anti-PC not being protective for early RA-patient. Another possibility may be that IL-6 inhibition reduces the levels of IgA anti-PC. This finding may imply on tocilizumab not having a positive effect in the long run in early-RA patients and the risk of developing atherosclerosis and CVD increases subsequently. This interesting finding needs further analysis.

Although this present study does not demonstrate the protective effects of IgA anti-PC at baseline, further studies with this cohort will be going on. Analysis of different immunoglobulin subgroups, IgM and IgG1 will be performed in the NORD-STAR cohort to establish a full analysis of the association. Also, analyses of subgroups in high levels, above 75^{th} and 90^{th} percentile of IgA anti-PC at baseline have not been done yet, which are planned for further analysis.

Some limitations in this study did occur. It would be interesting to investigate the mechanisms and association of the treatments in-depth with IgA anti-PC in RA-patients, which was not possible due to limited time. The patients were only followed up to 24 weeks and although a significant difference was observed in some arms, a longer follow up time of period would be better to determine if there is a significant association between disease activity and clinical outcome in the RA patients and its association to atherosclerosis. The NORD-STAR trial is in total 48 weeks long and it would be interesting to compare baseline and week 24 with week 48 and see its association with IgA anti-PC. Thus, a deeper study would be of great importance with pro-inflammatory mediators in cell culture and with different antibodies.

5. Conclusion

This present study offers new insights into the role of IgA anti-PC in the development of RA with different treatments. IgA anti-PC decreased during treatment, in the whole group. The results indicated that IgA anti-PC might be a novel biomarker for RA, as the association between IgA anti-PC and disease activity in RA was elucidated to some extent. Some treatment condition decreased the levels of IgA anti-PC, which might have an adverse effect on RA patients and may lead to a higher risk of developing atherosclerosis and CVD.

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7. References

1. National Academies of Sciences E, Division H and M, Services B on HC, Treatment C on IDMCL to I with. Musculoskeletal Disorders [Internet]. Selected Health Conditions and Likelihood of Improvement with Treatment. National Academies Press (US); 2020 [cited 2020 Dec 29]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559512/

2. Chauhan K, Jandu JS, Goyal A, Bansal P, Al-Dhahir MA. Rheumatoid Arthritis. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Dec 29]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK441999/

3. Rudan I, Sidhu S, Papana A, Meng S, Xin–Wei Y, Wang W, et al. Prevalence of rheumatoid arthritis in low– and middle–income countries: A systematic review and analysis. J Glob Health [Internet]. [cited 2020 Dec 29];5(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4416333/

4. Fang Q, Ou J, Nandakumar KS. Autoantibodies as Diagnostic Markers and Mediator of Joint Inflammation in Arthritis [Internet]. Vol. 2019, Mediators of Inflammation. Hindawi; 2019 [cited 2020 Dec 30]. p. e6363086. Available from: https://www.hindawi.com/journals/mi/2019/6363086/

5. Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al. Rheumatoid arthritis. Nat Rev Dis Primer. 2018 Feb 8;4(1):1–23.

6. Demoruelle MK, Deane KD, Holers VM. When and Where Does Inflammation Begin in Rheumatoid Arthritis? Curr Opin Rheumatol. 2014 Jan;26(1):64–71.

7. Information NC for B, Pike USNL of M 8600 R, MD B, Usa 20894. What is an inflammation? [Internet]. InformedHealth.org [Internet]. Institute for Quality and Efficiency in Health Care (IQWiG); 2018 [cited 2020 Dec 30]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279298/

8. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res [Internet]. 2018 Apr 27 [cited 2020 Dec 30];6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5920070/
9. Wang Q, Sun X. Recent advances in nanomedicines for the treatment of rheumatoid arthritis. Biomater Sci. 2017 Jul 26;5(8):1407–20.

10. Nalbant S, Birlik AM. Cytokines in Rheumatoid Arthritis (RA). New Dev Pathog Rheum Arthritis [Internet]. 2017 Feb 22 [cited 2020 Dec 30]; Available from: https://www.intechopen.com/books/new-developments-in-the-pathogenesis-of-rheumatoid-arthritis/cytokines-in-rheumatoid-arthritis-ra-

11. Cugno M, Ingegnoli F, Gualtierotti R, Fantini F. Potential Effect of Anti-Tumour Necrosis Factor-Alpha Treatment on Reducing the Cardiovascular Risk Related to Rheumatoid Arthritis. Curr Vasc Pharmacol. 2010 Feb 1;8:285–92.

12. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular Manifestations in Rheumatoid Arthritis. Mædica. 2010 Dec;5(4):286–91.

13. How citrullination invaded rheumatoid arthritis research | Arthritis Research & Therapy | Full Text [Internet]. [cited 2020 Dec 30]. Available from: https://arthritis-research.biomedcentral.com/articles/10.1186/ar4458

14. Kampstra ASB, Toes REM. HLA class II and rheumatoid arthritis: the bumpy road of revelation. Immunogenetics. 2017;69(8):597–603.

15. Yoshida M, Tsuji M, Kurosaka D, Kurosaka D, Yasuda J, Ito Y, et al. Autoimmunity to citrullinated type II collagen in rheumatoid arthritis. Mod Rheumatol. 2006 Oct;16(5):276–81.
16. Aarvak T, Natvig JB. Cell-cell interactions in synovitis: Antigen presenting cells and T cell interaction in rheumatoid arthritis. Arthritis Res Ther. 2000 Oct 27;3(1):13.

17. Frostegård J. Cardiovascular co-morbidity in patients with rheumatic diseases. Arthritis Res Ther. 2011;13(3):225.

18. Gerli R, Bocci EB, Sherer Y, Vaudo G, Moscatelli S, Shoenfeld Y. Association of anti-cyclic citrullinated peptide antibodies with subclinical atherosclerosis in patients with rheumatoid arthritis. Ann Rheum Dis. 2008 May 1;67(5):724–5.

19. López-Longo FJ, Oliver-Miñarro D, de la Torre I, González-Díaz de Rábago E, Sánchez-Ramón S, Rodríguez-Mahou M, et al. Association between anti-cyclic citrullinated peptide antibodies and ischemic heart disease in patients with rheumatoid arthritis. Arthritis Rheum. 2009 Apr 15;61(4):419–24.

20. Charles-Schoeman C. Cardiovascular Disease and Rheumatoid Arthritis: An Update. Curr Rheumatol Rep. 2012 Oct;14(5):455–62.

21. Rawla P. Cardiac and vascular complications in rheumatoid arthritis. Reumatologia. 2019;57(1):27–36.

22. Frostegård J. Low level natural antibodies against phosphorylcholine: A novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease. Clin Immunol. 2010 Jan 1;134(1):47–54.

23. Gaziano T, Reddy KS, Paccaud F, Horton S, Chaturvedi V. Cardiovascular Disease. In: Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Disease Control Priorities in Developing Countries [Internet]. 2nd ed. Washington (DC): World Bank; 2006 [cited 2020 Dec 30]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK11767/

24. Frostegård J. Immunity, atherosclerosis and cardiovascular disease. BMC Med. 2013 May 1;11:117.

25. Frostegard J. 45 - AUTOANTIBODIES IN ATHEROSCLEROSIS. In: Shoenfeld Y, Gershwin ME, Meroni PL, editors. Autoantibodies (Second Edition) [Internet]. Burlington: Elsevier; 2007 [cited 2020 Dec 30]. p. 341–7. Available from: http://www.sciencedirect.com/science/article/pii/B9780444527639500494

26. Levitan I, Volkov S, Subbaiah PV. Oxidized LDL: Diversity, Patterns of Recognition, and Pathophysiology. Antioxid Redox Signal. 2010 Jul 1;13(1):39–75.

27. Hardingham T. CHAPTER 5 - Proteoglycans and Glycosaminoglycans. In: Seibel MJ, Robins SP, Bilezikian JP, editors. Dynamics of Bone and Cartilage Metabolism (Second Edition) [Internet]. Burlington: Academic Press; 2006 [cited 2020 Dec 30]. p. 85–98. Available from: http://www.sciencedirect.com/science/article/pii/B9780120885626500066

28. Rhoads JP, Major AS. How Oxidized Low-Density Lipoprotein Activates Inflammatory Responses. Crit Rev Immunol. 2018;38(4):333–42.

29. Hansson GK, Robertson A-KL, Söderberg-Nauclér C. Inflammation and atherosclerosis. Annu Rev Pathol Mech Dis. 2006 Jan 24;1(1):297–329.

30. Linton MF, Yancey PG, Davies SS, Jerome WG, Linton EF, Song WL, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000 [cited 2020 Dec 30]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK343489/

31. Seo J-W, Yang E-J, Yoo K-H, Choi I-H. Macrophage Differentiation from Monocytes Is Influenced by the Lipid Oxidation Degree of Low Density Lipoprotein. Mediators Inflamm [Internet]. 2015 [cited 2020 Dec 30];2015. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4532889/

32. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature. 2011 May;473(7347):317–25.

33. Doran Amanda C., Meller Nahum, McNamara Coleen A. Role of Smooth Muscle Cells in the Initiation and Early Progression of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2008 May 1;28(5):812–9.

34. Samal SK, Qureshi AR, Rahman M, Stenvinkel P, Frostegård J. Different subclasses and isotypes of antibodies against phosphorylcholine in haemodialysis patients: association with mortality. Clin Exp Immunol. 2020;201(1):94–104.

35. Ashraf MA, Nookala V. Biochemistry, Platelet Activating Factor. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Dec 30]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK557392/

36. Fiskesund R, Steen J, Amara K, Murray F, Szwajda A, Liu A, et al. Naturally Occurring Human Phosphorylcholine Antibodies Are Predominantly Products of Affinity-Matured B Cells in the Adult. J Immunol. 2014 May 15;192(10):4551–9.

37. Emery P, Salmon M. Early rheumatoid arthritis: time to aim for remission? Ann Rheum Dis. 1995 Dec 1;54(12):944–7.

38. Bullock J, Rizvi SAA, Saleh AM, Ahmed SS, Do DP, Ansari RA, et al. Rheumatoid Arthritis: A Brief Overview of the Treatment. Med Princ Pract. 2019 Mar;27(6):501–7.

39. Crofford LJ. Use of NSAIDs in treating patients with arthritis. Arthritis Res Ther. 2013;15(Suppl 3):S2.

40. Bijlsma JWJ, Boers M, Saag KG, Furst DE. Glucocorticoids in the treatment of early and late RA. Ann Rheum Dis. 2003 Nov 1;62(11):1033–7.

41. Benjamin O, Bansal P, Goyal A, Lappin SL. Disease Modifying Anti-Rheumatic Drugs (DMARD). In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 [cited 2020 Dec 30]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK507863/

42. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol. 2020 Mar;16(3):145–54.

43. Kaneko A. Tocilizumab in rheumatoid arthritis: efficacy, safety and its place in therapy. Ther Adv Chronic Dis. 2013 Jan;4(1):15–21.

44. Schrezenmeier E, Dörner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol. 2020 Mar;16(3):155–66.

45. Köhler BM, Günther J, Kaudewitz D, Lorenz H-M. Current Therapeutic Options in the Treatment of Rheumatoid Arthritis. J Clin Med [Internet]. 2019 Jun 28 [cited 2020 Dec 30];8(7). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6678427/

46. Ruiz Garcia V, Burls A, Cabello JB, Vela Casasempere P, Bort-Marti S, Bernal JA. Certolizumab pegol (CDP870) for rheumatoid arthritis in adults. Cochrane Database Syst Rev. 2017 Sep 8;9:CD007649.

47. Frostegård J. Cardiovascular co-morbidity in patients with rheumatic diseases. Arthritis Res Ther. 2011;13(3):225.

48. Vital EM, Emery P. Abatacept in the treatment of rheumatoid arthritis. Ther Clin Risk Manag. 2006 Dec;2(4):365.

49. Jensen Hansen IM, Asmussen Andreasen R, van Bui Hansen MN, Emamifar A. The Reliability of Disease Activity Score in 28 Joints–C-Reactive Protein Might Be Overestimated in a Subgroup of Rheumatoid Arthritis Patients, When the Score Is Solely Based on Subjective Parameters. J Clin Rheumatol. 2017 Mar;23(2):102–6.

50. Thiagarajan D, Oparina N, Lundström S, Zubarev R, Sun J, Alarcon-Riquelme M, et al. IgM antibodies against malondialdehyde and phosphorylcholine in different systemic rheumatic diseases. Sci Rep. 2020 Jul 3;10(1):11010.

51. Fiskesund R, Su J, Bulatovic I, Vikström M, de Faire U, Frostegård J. IgM phosphorylcholine antibodies inhibit cell death and constitute a strong protection marker for atherosclerosis development, particularly in combination with other auto-antibodies against modified LDL. Results Immunol. 2012 Jan 1;2:13–8.

52. Samal SK, Qureshi AR, Rahman M, Stenvinkel P, Frostegård J. Antibodies against Malondialdehyde in Haemodialysis Patients and Its Association with Clinical Outcomes: Differences between Subclasses and Isotypes. J Clin Med. 2020 Mar;9(3):753.

53. Frostegård J. Low level natural antibodies against phosphorylcholine: A novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease. Clin Immunol. 2010 Jan 1;134(1):47–54.

54. Houge IS, Hoff M, Thomas R, Videm V. Mortality is increased in patients with rheumatoid arthritis or diabetes compared to the general population – the Nord-Trøndelag Health Study. Sci Rep. 2020 Feb 27;10(1):3593.

55. Su J, Georgiades A, Wu R, Thulin T, Faire U de, Frostegård J. Antibodies of IgM subclass to phosphorylcholine and oxidized LDL are protective factors for atherosclerosis in patients with hypertension. Atherosclerosis. 2006 Sep 1;188(1):160–6.

56. Ajeganova S. Addressing cardiovascular risk factors and therapy effects in rheumatoid arthritis: implications for atherosclerosis and cardiovascular disease. [Stockholm]: Karolinska institutet; 2012.

57. Harnett W, Harnett MM. Phosphorylcholine: friend or foe of the immune system? Immunol Today. 1999 Mar 1;20(3):125–9.

58. Hafström I, Rohani M, Deneberg S, Wörnert M, Jogestrand T, Frostegård J. Effects of low-dose prednisolone on endothelial function, atherosclerosis, and traditional risk factors for atherosclerosis in patients with rheumatoid arthritis--a randomized study. J Rheumatol. 2007 Sep;34(9):1810–6.

59. Cavagna L, Boffini N, Cagnotto G, Inverardi F, Grosso V, Caporali R. Atherosclerosis and Rheumatoid Arthritis: More Than a Simple Association [Internet]. Vol. 2012, Mediators of Inflammation. Hindawi; 2012 [cited 2020 Dec 30]. p. e147354. Available from: https://www.hindawi.com/journals/mi/2012/147354/

60. Active conventional treatment and three different biological treatments in early rheumatoid arthritis: phase IV investigator initiated, randomised, observer blinded clinical trial | The BMJ [Internet]. [cited 2021 Jan 11]. Available from: https://www.bmj.com/content/371/bmj.m4328/rapid-responses

8. Appendix

8.1. List of material used

8.1.1. Instruments

800 TS Absorbance Reader	Biotek Instruments Inc.	Winooski, Vermont, United States
ELx50^{TM} Microplate Strip Washer	Biotek Instruments Inc.	Winooski, Vermont, United States

8.1.2. Reagents

Bovine serum albumin (BSA)	Sigma-Aldrich	St. Louis, Missouri, United States
Phosphorylcholine-bovine serum albumin (PC-BSA)		
Phosphate buffered saline (PBS) 1x	Thermo Fisher scientific	Waltham, Massachusetts, United States
Phosphate buffered saline (PBS) 20x	Santa Cruz Biotechnology	Dallas, Texas, United States
Streptavidin	Thermo Fisher scientific	Waltham, Massachusetts, United States
3,3',5,5'-Tetramethylbenzadine (TMB)	Thermo Fisher scientific	Waltham, Massachusetts, United States
Tween20	Sigma-Aldrich	St. Louis, Missouri, United States

8.1.3. Buffers

Stop solution	1N H_2SO_4 in ultrapure H_2O
Wash buffer	0,05% Tween20 1x PBS (diluted from 20x PBS) Deionized H2O

8.2. Supplementary data

8.2.1. Experimental data

Supplementary table 1: Levels of IgA anti-PC in baseline (BL) and after 24 weeks (W24) in NORD-STAR patients.

Number of values	**341** (BL1)	**341** (W24)
Minimum	12,21	11,79
25% Percentile	84,38	76,66
Median	101,1	94,84
75% Percentile	120,2	116,9
Maximum	182,1	207,7
Range	169,9	195,9
10% Percentile	69,76	64,15
90% Percentile	141,3	135,7
95% CI of median		
Actual confidence level	96,05%	96,05%
Lower confidence limit	97,95	90,81
Upper confidence limit	104,5	98,01
Mean	103,2	97,44
Std. Deviation	26,87	29,11
Std. Error of Mean	1,455	1,576
Lower 95% CI of mean	100,4	94,34
Upper 95% CI of mean	106,1	100,5
Coefficient of variation	26,02%	29,87%
Geometric mean	99,44	92,70
Geometric SD factor	1,335	1,396
Lower 95% CI of geo. mean	96,43	89,47
Upper 95% CI of geo. mean	102,5	96,06
Harmonic mean	94,56	86,75
Lower 95% CI of harm. mean	90,13	82,34
Upper 95% CI of harm. mean	99,44	91,66
Quadratic mean	106,7	101,7
Lower 95% CI of quad. mean	103,8	98,44
Upper 95% CI of quad. mean	109,5	104,8
Skewness	0,1884	0,3300
Kurtosis	-0,01305	0,4704
Sum	35207	33226

Supplementary table 2: DAS28 and IgA anti-PC association in baseline (BL) below 10th percentile and above 90th percentile

	LOW 10	HIGH 90
Number of values	33	313
Minimum	66,97	12,21
25% Percentile	91,36	84,16
Median	103,3	101,3
75% Percentile	126,3	121,0
Maximum	169,3	182,1
Range	102,3	169,9
10% Percentile	83,62	69,64
90% Percentile	145,5	141,7
95% CI of median		
Actual confidence level	96,49%	95,83%
Lower confidence limit	94,79	98,12
Upper confidence limit	120,0	105,2
Mean	109,5	103,2
Std. Deviation	24,58	27,16
Std. Error of Mean	4,279	1,535
Lower 95% CI of mean	100,8	100,2
Upper 95% CI of mean	118,2	106,2
Coefficient of variation	22,45%	26,32%
Geometric mean	106,9	99,29
Geometric SD factor	1,248	1,342
Lower 95% CI of geo. mean	98,80	96,10
Upper 95% CI of geo. mean	115,6	102,6
Harmonic mean	104,4	94,20
Lower 95% CI of harm. mean	96,84	89,47
Upper 95% CI of harm. mean	113,2	99,45
Quadratic mean	112,1	106,7
Lower 95% CI of quad. mean	102,7	103,6
Upper 95% CI of quad. mean	120,8	109,7
Skewness	0,5327	0,1544
Kurtosis	-0,3913	-0,06296
Sum	3612	32306

Supplementary table 3: DAS28 (CRP mg/L) in baseline (BL) and after 24 weeks (W24)

	BL	W 24
Number of values	340	340
Minimum	2,220	1,030
25% Percentile	4,353	1,470
Median	5,100	1,990
75% Percentile	5,820	2,830
Maximum	8,300	5,040
Range	6,080	4,010
10% Percentile	3,872	1,200
90% Percentile	6,529	3,479
95% CI of median		
Actual confidence level	95,54%	95,54%
Lower confidence limit	4,960	1,860
Upper confidence limit	5,210	2,120
Mean	5,131	2,208
Std. Deviation	1,027	0,9022
Std. Error of Mean	0,05567	0,04893
Lower 95% CI of mean	5,021	2,112
Upper 95% CI of mean	5,240	2,304
Coefficient of variation	20,01%	40,86%
Geometric mean	5,026	2,042
Geometric SD factor	1,229	1,481
Lower 95% CI of geo. mean	4,917	1,958
Upper 95% CI of geo. mean	5,138	2,129
Harmonic mean	4,918	1,897
Lower 95% CI of harm. mean	4,806	1,824
Upper 95% CI of harm. mean	5,035	1,975
Quadratic mean	5,232	2,385
Lower 95% CI of quad. mean	5,121	2,275
Upper 95% CI of quad. mean	5,341	2,490
Skewness	0,1962	0,8856
Kurtosis	-0,1991	0,1393
Sum	1744	750,7

Supplementary table 4: DAS28 with anti-PC in week.24 (W24)

	Das<2.6	Das>2.6
Number of values	239	100
Minimum	23,19	11,79
25% Percentile	78,62	73,30
Median	94,94	92,88
75% Percentile	117,4	113,5
Maximum	207,7	155,7
Range	184,5	143,9
10% Percentile	64,21	61,13
90% Percentile	138,7	134,6
95% CI of median		
Actual confidence level	96,18%	96,48%
Lower confidence limit	89,91	88,43
Upper confidence limit	98,01	102,0
Mean	98,31	94,58
Std. Deviation	29,60	28,28
Std. Error of Mean	1,914	2,828
Lower 95% CI of mean	94,54	88,97
Upper 95% CI of mean	102,1	100,2
Coefficient of variation	30,10%	29,90%
Geometric mean	93,75	89,42
Geometric SD factor	1,374	1,450
Lower 95% CI of geo. mean	90,03	83,06
Upper 95% CI of geo. mean	97,63	96,25
Harmonic mean	88,80	81,46
Lower 95% CI of harm. mean	84,74	71,68
Upper 95% CI of harm. mean	93,26	94,33
Quadratic mean	102,7	98,68
Lower 95% CI of quad. mean	98,60	93,12
Upper 95% CI of quad. mean	106,6	103,9
Skewness	0,5090	-0,1054
Kurtosis	0,5736	-0,06631
Sum	23497	9458

Supplementary table 5: DAS28 and IgA Anti-PC association in baseline (BL).

	HIGH 10	LOW 90
Number of values	31	315
Minimum	67,52	12,21
25% Percentile	86,49	84,50
Median	111,0	100,8
75% Percentile	139,5	120,4
Maximum	156,4	182,1
Range	88,90	169,9
10% Percentile	69,34	69,92
90% Percentile	149,6	141,2
95% CI of median		
Actual confidence level	97,06%	95,76%
Lower confidence limit	94,14	97,89
Upper confidence limit	127,8	104,3
Mean	111,8	103,0
Std. Deviation	27,85	26,89
Std. Error of Mean	5,002	1,515
Lower 95% CI of mean	101,5	100,1
Upper 95% CI of mean	122,0	106,0
Coefficient of variation	24,92%	26,10%
Geometric mean	108,2	99,21
Geometric SD factor	1,300	1,338
Lower 95% CI of geo. mean	98,30	96,06
Upper 95% CI of geo. mean	119,2	102,5
Harmonic mean	104,6	94,21
Lower 95% CI of harm. mean	95,07	89,52
Upper 95% CI of harm. mean	116,2	99,42
Quadratic mean	115,1	106,5
Lower 95% CI of quad. mean	104,7	103,4
Upper 95% CI of quad. mean	124,6	109,5
Skewness	-0,04279	0,1915
Kurtosis	-1,177	0,07341
Sum	3464	32460

Supplementary table 6: Association of DAS28 with IgA anti-PC in week 24 below10th percentile and above 90th percentile.

	LOW 10	HIGH 90
Number of values	33	310
Minimum	37,09	11,79
25% Percentile	73,42	76,97
Median	87,45	95,47
75% Percentile	103,9	117,4
Maximum	161,0	207,7
Range	123,9	195,9
10% Percentile	52,18	65,28
90% Percentile	131,5	137,4
95% CI of median		
Actual confidence level	96,49%	95,33%
Lower confidence limit	76,60	92,46
Upper confidence limit	99,85	98,08
Mean	89,17	98,27
Std. Deviation	28,21	29,15
Std. Error of Mean	4,911	1,656
Lower 95% CI of mean	79,16	95,01
Upper 95% CI of mean	99,17	101,5
Coefficient of variation	31,64%	29,67%
Geometric mean	84,69	93,55
Geometric SD factor	1,397	1,395
Lower 95% CI of geo. mean	75,22	90,13
Upper 95% CI of geo. mean	95,35	97,09
Harmonic mean	80,01	87,50
Lower 95% CI of harm. mean	70,81	82,73
Upper 95% CI of harm. mean	91,95	92,85
Quadratic mean	93,39	102,5
Lower 95% CI of quad. mean	82,43	99,08
Upper 95% CI of quad. mean	103,2	105,8
Skewness	0,4033	0,3339
Kurtosis	0,2029	0,5067
Sum	2942	30463

Supplementary table 7: Association of DAS28 with IgA anti-PC in week 24 below 90th percentile and above 10th percentile.

	LOW 90	HIGH 10
Number of values	313	32
Minimum	11,79	29,46
25% Percentile	79,03	70,05
Median	95,42	88,82
75% Percentile	117,3	110,2
Maximum	207,7	142,4
Range	195,9	113,0
10% Percentile	64,97	47,86
90% Percentile	137,6	127,6
95% CI of median		
Actual confidence level	95,83%	97,99%
Lower confidence limit	92,46	74,17
Upper confidence limit	98,08	104,8
Mean	98,50	89,04
Std. Deviation	29,17	27,69
Std. Error of Mean	1,649	4,895
Lower 95% CI of mean	95,25	79,06
Upper 95% CI of mean	101,7	99,02
Coefficient of variation	29,61%	31,10%
Geometric mean	93,82	84,30
Geometric SD factor	1,391	1,425
Lower 95% CI of geo. mean	90,44	74,20
Upper 95% CI of geo. mean	97,32	95,78
Harmonic mean	87,88	78,77
Lower 95% CI of harm. mean	83,17	68,18
Upper 95% CI of harm. mean	93,15	93,27
Quadratic mean	102,7	93,12
Lower 95% CI of quad. mean	99,31	82,92
Upper 95% CI of quad. mean	106,0	102,3
Skewness	0,3555	-0,05081
Kurtosis	0,4908	-0,4432
Sum	30829	2849

Supplementary table 8: Comparison of treatment with ARM 1 in BL and W24 with IgA anti-PC levels.

	ARM 1 BL	ARM 1 W24
Number of values	84	82
Minimum	46,43	23,19
25% Percentile	80,29	73,85
Median	93,82	89,23
75% Percentile	117,7	114,1
Maximum	169,3	161,0
Range	122,8	137,8
10% Percentile	66,83	60,41
90% Percentile	140,1	137,8
95% CI of median		
Actual confidence level	96,25%	96,48%
Lower confidence limit	88,66	81,79
Upper confidence limit	100,4	98,08
Mean	98,99	93,15
Std. Deviation	28,13	28,72
Std. Error of Mean	3,070	3,171
Lower 95% CI of mean	92,88	86,84
Upper 95% CI of mean	105,1	99,46
Coefficient of variation	28,42%	30,83%
Geometric mean	94,98	88,28
Geometric SD factor	1,342	1,417
Lower 95% CI of geo. mean	89,10	81,77
Upper 95% CI of geo. mean	101,2	95,30
Harmonic mean	90,87	82,40
Lower 95% CI of harm. mean	85,06	74,91
Upper 95% CI of harm. mean	97,53	91,56
Quadratic mean	102,9	97,43
Lower 95% CI of quad. mean	96,39	90,90
Upper 95% CI of quad. mean	109,0	103,5
Skewness	0,3816	0,1657
Kurtosis	-0,2887	-0,1252
Sum	8315	7639

Supplementary table 9: Comparison of treatment with ARM 2 in BL and W24 with IgA anti-PC levels.

	ARM 2 BL	ARM 2 W24
Number of values	87	88
Minimum	52,15	36,25
25% Percentile	88,90	88,87
Median	104,3	105,2
75% Percentile	120,5	126,2
Maximum	161,2	194,7
Range	109,0	158,5
10% Percentile	69,42	67,25
90% Percentile	129,6	144,6
95% CI of median		
Actual confidence level	96,86%	95,78%
Lower confidence limit	99,03	96,80
Upper confidence limit	111,7	112,0
Mean	104,2	106,8
Std. Deviation	23,29	30,14
Std. Error of Mean	2,497	3,213
Lower 95% CI of mean	99,28	100,4
Upper 95% CI of mean	109,2	113,2
Coefficient of variation	22,35%	28,22%
Geometric mean	101,5	102,4
Geometric SD factor	1,266	1,354
Lower 95% CI of geo. mean	96,55	96,01
Upper 95% CI of geo. mean	106,8	109,2
Harmonic mean	98,65	97,47
Lower 95% CI of harm. mean	93,55	90,68
Upper 95% CI of harm. mean	104,3	105,4
Quadratic mean	106,8	110,9
Lower 95% CI of quad. mean	101,7	104,2
Upper 95% CI of quad. mean	111,6	117,3
Skewness	0,08464	0,3442
Kurtosis	0,05440	0,4114
Sum	9069	9399

Supplementary table 10: Comparison of treatment with ARM 3 in BL and W24 with IgA anti-PC levels.

	ARM 3 BL	ARM 3 W24
Number of values	92	91
Minimum	57,46	11,79
25% Percentile	91,22	79,80
Median	105,5	94,27
75% Percentile	123,5	117,5
Maximum	182,1	207,7
Range	124,6	195,9
10% Percentile	72,50	67,16
90% Percentile	143,6	137,1
95% CI of median		
Actual confidence level	95,30%	96,46%
Lower confidence limit	100,3	89,48
Upper confidence limit	111,7	104,8
Mean	108,3	98,70
Std. Deviation	26,62	28,50
Std. Error of Mean	2,776	2,987
Lower 95% CI of mean	102,8	92,76
Upper 95% CI of mean	113,8	104,6
Coefficient of variation	24,59%	28,87%
Geometric mean	105,1	93,98
Geometric SD factor	1,282	1,414
Lower 95% CI of geo. mean	99,78	87,44
Upper 95% CI of geo. mean	110,6	101,0
Harmonic mean	101,9	86,27
Lower 95% CI of harm. mean	96,74	75,08
Upper 95% CI of harm. mean	107,5	101,4
Quadratic mean	111,5	102,7
Lower 95% CI of quad. mean	105,6	96,24
Upper 95% CI of quad. mean	117,0	108,8
Skewness	0,4297	0,4458
Kurtosis	-0,1272	1,750
Sum	9961	8982

Supplementary table 11: Comparison of treatment with ARM 4 in BL and W24 with IgA anti-PC levels.

	ARM 4 BL	ARM 4 W24
Number of values	84	82
Minimum	12,21	37,09
25% Percentile	80,73	70,33
Median	101,2	86,56
75% Percentile	120,8	111,8
Maximum	159,9	152,1
Range	147,7	115,1
10% Percentile	67,74	56,23
90% Percentile	143,0	132,9
95% CI of median		
Actual confidence level	96,25%	96,48%
Lower confidence limit	91,12	81,14
Upper confidence limit	107,1	94,17
Mean	100,9	90,15
Std. Deviation	28,72	27,06
Std. Error of Mean	3,133	2,988
Lower 95% CI of mean	94,65	84,20
Upper 95% CI of mean	107,1	96,10
Coefficient of variation	28,47%	30,02%
Geometric mean	95,77	86,01
Geometric SD factor	1,436	1,371
Lower 95% CI of geo. mean	88,53	80,25
Upper 95% CI of geo. mean	103,6	92,18
Harmonic mean	87,19	81,73
Lower 95% CI of harm. mean	75,16	76,03
Upper 95% CI of harm. mean	103,8	88,35
Quadratic mean	104,8	94,08
Lower 95% CI of quad. mean	98,66	87,85
Upper 95% CI of quad. mean	110,7	99,91
Skewness	-0,1475	0,2875
Kurtosis	0,1477	-0,5933
Sum	8474	7392

Supplementary table 12: IgA anti-PC levels of different treatments (ARM 1; ARM 2; ARM 3; ARM 4) in week baseline.

	ARM 1	ARM 2	ARM 3	ARM 4
Number of values	84	87	92	84
Minimum	46,43	52,15	57,46	12,21
25% Percentile	80,29	88,90	91,22	80,73
Median	93,82	104,3	105,5	101,2
75% Percentile	117,7	120,5	123,5	120,8
Maximum	169,3	161,2	182,1	159,9
Range	122,8	109,0	124,6	147,7
10% Percentile	66,83	69,42	72,50	67,74
90% Percentile	140,1	129,6	143,6	143,0
95% CI of median				
Actual confidence level	96,25%	96,86%	95,30%	96,25%
Lower confidence limit	88,66	99,03	100,3	91,12
Upper confidence limit	100,4	111,7	111,7	107,1
Mean	98,99	104,2	108,3	100,9
Std. Deviation	28,13	23,29	26,62	28,72
Std. Error of Mean	3,070	2,497	2,776	3,133
Lower 95% CI of mean	92,88	99,28	102,8	94,65
Upper 95% CI of mean	105,1	109,2	113,8	107,1
Coefficient of variation	28,42%	22,35%	24,59%	28,47%
Geometric mean	94,98	101,5	105,1	95,77
Geometric SD factor	1,342	1,266	1,282	1,436
Lower 95% CI of geo. mean	89,10	96,55	99,78	88,53
Upper 95% CI of geo. mean	101,2	106,8	110,6	103,6
Harmonic mean	90,87	98,65	101,9	87,19
Lower 95% CI of harm. mean	85,06	93,55	96,74	75,16
Upper 95% CI of harm. mean	97,53	104,3	107,5	103,8
Quadratic mean	102,9	106,8	111,5	104,8
Lower 95% CI of quad. mean	96,39	101,7	105,6	98,66
Upper 95% CI of quad. mean	109,0	111,6	117,0	110,7
Skewness	0,3816	0,08464	0,4297	-0,1475
Kurtosis	-0,2887	0,05440	-0,1272	0,1477
Sum	8315	9069	9961	8474

Supplementary table 13: IgA anti-PC levels of different treatments (ARM 1; ARM 2; ARM 3; ARM 4) in week 24.

	ARM 1	ARM 2	ARM 3	ARM 4
Number of values	82	88	91	82
Minimum	23,19	36,25	11,79	37,09
25% Percentile	73,85	88,87	79,80	70,33
Median	89,23	105,2	94,27	86,56
75% Percentile	114,1	126,2	117,5	111,8
Maximum	161,0	194,7	207,7	152,1
Range	137,8	158,5	195,9	115,1
10% Percentile	60,41	67,25	67,16	56,23
90% Percentile	137,8	144,6	137,1	132,9
95% CI of median				
Actual confidence level	96,48%	95,78%	96,46%	96,48%
Lower confidence limit	81,79	96,80	89,48	81,14
Upper confidence limit	98,08	112,0	104,8	94,17
Mean	93,15	106,8	98,70	90,15
Std. Deviation	28,72	30,14	28,50	27,06
Std. Error of Mean	3,171	3,213	2,987	2,988
Lower 95% CI of mean	86,84	100,4	92,76	84,20
Upper 95% CI of mean	99,46	113,2	104,6	96,10
Coefficient of variation	30,83%	28,22%	28,87%	30,02%
Geometric mean	88,28	102,4	93,98	86,01
Geometric SD factor	1,417	1,354	1,414	1,371
Lower 95% CI of geo. mean	81,77	96,01	87,44	80,25
Upper 95% CI of geo. mean	95,30	109,2	101,0	92,18
Harmonic mean	82,40	97,47	86,27	81,73
Lower 95% CI of harm. mean	74,91	90,68	75,08	76,03
Upper 95% CI of harm. mean	91,56	105,4	101,4	88,35
Quadratic mean	97,43	110,9	102,7	94,08
Lower 95% CI of quad. mean	90,90	104,2	96,24	87,85
Upper 95% CI of quad. mean	103,5	117,3	108,8	99,91
Skewness	0,1657	0,3442	0,4458	0,2875
Kurtosis	-0,1252	0,4114	1,750	-0,5933
Sum	7639	9399	8982	7392

Supplementary table 14: Comparison of IgA anti-PC levels and DAS28 in BL and W24

	DAS-BL <2.6	DAS-BL>2.6	DAS-W24 <2.6	DAS-W24 >2.6
Number of values	1	345	241	104
Minimum	122,9	12,21	23,19	11,79
25% Percentile	122,9	84,51	77,83	74,07
Median	122,9	101,3	94,34	93,74
75% Percentile	122,9	120,7	117,3	114,3
Maximum	122,9	182,1	207,7	155,7
Range	0,000	169,9	184,5	143,9
10% Percentile	122,9	69,81	64,13	62,11
90% Percentile	122,9	141,6	138,5	134,6
95% CI of median				
Actual confidence level	5,000%	95,94%	96,09%	96,10%
Lower confidence limit		98,12	89,48	88,76
Upper confidence limit		104,8	97,85	104,1
Mean	122,9	103,4	98,09	95,27
Std. Deviation	0,000	26,93	29,61	28,11
Std. Error of Mean	0,000	1,450	1,907	2,757
Lower 95% CI of mean		100,6	94,34	89,81
Upper 95% CI of mean		106,3	101,9	100,7
Coefficient of variation	0,000%	26,04%	30,19%	29,51%
Geometric mean	122,9	99,62	93,52	90,18
Geometric SD factor	1,000	1,335	1,375	1,444
Lower 95% CI of geo. mean		96,62	89,82	83,96
Upper 95% CI of geo. mean		102,7	97,38	96,86
Harmonic mean	122,9	94,73	88,57	82,29
Lower 95% CI of harm. mean		90,33	84,54	72,64
Upper 95% CI of harm. mean		99,58	93,00	94,90
Quadratic mean	122,9	106,9	102,4	99,30
Lower 95% CI of quad. mean	0,000	104,0	98,41	93,91
Upper 95% CI of quad. mean		109,7	106,3	104,4
Skewness		0,1828	0,5130	-0,1415
Kurtosis		-0,04234	0,5707	-0,05810
Sum	122,9	35684	23640	9909