# The alternative complement pathway in ANCA-associated vasculitis: further evidence and a meta-analysis

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**Abstract**

Objectives. We compared the common pathway components C3a, C5a and membrane attack complex (MAC), also known as C5b-9, and the alternative pathway components factor B and properdin in patients with ANCA-associated vasculitis (AAV) and healthy controls, and conducted a meta-analysis of the available clinical evidence for the role of complement activation in the pathogenesis of AAV. Methods. Complement components were evaluated in 59 patients with newly diagnosed or relapsing granulomatosis with polyangiitis or microscopic polyangiitis and 36 healthy volunteers. In 28 patients, testing was repeated in remission. Next, we performed a meta-analysis by searching databases to identify studies comparing complement levels in AAV patients and controls. A random-effects model was used for statistical analyses. Results. The median concentrations of MAC, C5a, C3a, and factor B were higher in active AAV patients ($p<0.001$). Achievement of remission was associated with reductions in C3a ($p=0.005$), C5a ($p=0.035$), and factor B levels ($p=0.045$), whereas MAC and properdin levels did not change. In active AAV, there were no effects of ANCA specificity, disease phenotype, previous immunosuppression, or disease severity on complement levels. A total of 1122 articles were screened, and five studies, including this report, were entered in the meta-analysis. Plasma MAC, C5a, and factor B in patients with active AAV were increased compared to patients in remission (excluding factor B) and controls. Changes in C3a were of borderline significance. Conclusion. Our findings and the results of the meta-analysis support activation of the complement system predominantly via the alternative pathway in AAV patients.

**Title page**

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**Objectives.** We compared the common pathway components C3a, C5a and membrane attack complex (MAC), also known as C5b-9, and the alternative pathway components factor B and properdin in patients with ANCA-associated vasculitis (AAV) and healthy controls, and conducted a meta-analysis of the available clinical evidence for the role of complement activation in the pathogenesis of AAV.

**Methods.** Complement components were evaluated in 59 patients with newly diagnosed or relapsing granulomatosis with polyangiitis or microscopic polyangiitis and 36 healthy volunteers. In 28 patients, testing was repeated in remission. Next, we performed a meta-analysis by searching databases to identify studies comparing complement levels in AAV patients and controls. A random-effects model was used for statistical analyses.

**Results.** The median concentrations of MAC, C5a, C3a, and factor B were higher in active AAV patients ($p<0.001$). Achievement of remission was associated with reductions in C3a ($p=0.005$), C5a ($p=0.035$), and factor B levels ($p=0.045$), whereas MAC and properdin levels did not change. In active AAV, there were no effects of ANCA specificity, disease phenotype, previous immunosuppression, or disease severity on complement levels.

A total of 1122 articles were screened, and five studies, including this report, were entered in the meta-analysis. Plasma MAC, C5a, and factor B in patients with active AAV were increased compared to patients in remission (excluding factor B) and controls. Changes in C3a were of borderline significance.

**Conclusion.** Our findings and the results of the meta-analysis support activation of the complement system predominantly via the alternative pathway in AAV patients.

**Keywords**

Complement, ANCA, vasculitis, biomarker, meta-analysis.

## Introduction

Granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA) belong to a group of systemic vasculitides characterized by necrotizing inflammation of small to medium-sized blood vessels. Antineutrophil cytoplasmic antibody (ANCA) in the circulation are present in a majority of patients with generalized disease. ANCA target neutrophil cytoplasmic constituents, in particular proteinase 3 (PR3) and myeloperoxidase (MPO), cause activation of neutrophils that release inflammatory cytokines, reactive oxygen species and lytic enzymes and induce excessive formation of neutrophil extracellular traps (NETs) [1]. These mechanisms underline, at least in part, the pathogenicity of ANCA and their involvement in the development of inflammation and injury of endothelial vascular cells. Disease manifestations are usually characterized by a pauci-immune phenomenon as biopsy finding with little, if any, immunoglobulin and complement deposition in the vessel walls. Complement C3 and C4 levels measured in the blood are in general normal.

However, recent experimental and clinical evidence suggests that activation of the alternative complement pathway is crucial in the pathogenesis of AAV [2, 3]. In 2007, Xiao et al. published the first study that highlighted a role of the complement system in the development of AAV [4]. In an animal model of pauci-immune crescentic glomerulonephritis induced by a single injection of anti-MPO IgG, the complement activation pathways were studied using mice with knockout of the common pathway component C5, classic and lectin binding pathway component C4, and alternative pathway component factor B. C5 and factor B deficient mice were found to be resistant to the development of crescentic glomerulonephritis, whereas deficiency of

C4 that is required for activation of C3 convertase and C5 convertase via both classic and lectin pathways did not prevent the disease. On the contrary, Hilhorst et al. found C4d in the majority of renal biopsies, suggesting classical pathway activation as well [5]. Incubation of MPO-ANCA or PR3-ANCA, unlike IgG from healthy controls, with human neutrophils was associated with release of factors that activated complement. These findings suggested that ANCA can cause an amplification loop between neutrophils and complement, that is, ANCA-induced activation of neutrophils leading to the generation of C5a, which in turn enhances neutrophil recruitment and priming and amplifies the inflammatory response [2, 6].

The role of the complement system in the development of AAV was further substantiated by clinical studies that analyzed various complement components in patients during different states of disease activity and healthy controls [7-10]. However, these studies were performed with a relatively small number of patients. Therefore, we aimed to compare the common pathway components C3a, C5a and membrane attack complex (MAC), also known as C5b-9, and the alternate pathway components factor B and properdin in patients with AAV and healthy controls, and to conduct a meta-analysis of the available clinical evidence for the role of complement activation in the pathogenesis of AAV.

## Material and methods

*Original study*

For inclusion in the study, we selected patients with newly diagnosed or relapsing AAV, who had a Birmingham Vasculitis Activity Score version 3 (BVAS v. 3) of [?]3. Diagnosis of granulomatosis with polyangiitis (GPA) or microscopic polyangiitis (MPA) was established according to the American College of Rheumatology criteria [11], EMA algorithm [12] and the Chapel Hill Conference Consensus definition [13]. Healthy control subjects were recruited at Tareev Clinic of Internal Disease (Moscow) and Vladimir Regional Clinical Hospital. All subjects provided written informed consent approved by the local ethics committee of the Sechenov University. The study was in compliance with the Declaration of Helsinki Principles.

Plasma concentrations of human complement components were determined by enzyme-linked immunosorbent assay, including MAC (HK328-02, Hycult Biotech, the Netherlands), C5a (HK349-01, Hycult Biotech, the Netherlands), C3a (HK354-01, Hycult Biotech, the Netherlands), factor B (EF7001-1, ASSAYPRO, USA), and properdin (factor P; SEA783Hu, Cloud-Clone Corporation, USA). All the complement components were assayed according to the manufacturer's instructions. Upper reference limits (the $97.5^{\text{th}}$ percentile) for each complement component were defined from the values in the control group after log transformation of the primary data.

Renal biopsies were evaluated according to a standardized protocol [14]. ANCA-associated glomerulonephritis class was established according to the Berden classification [15]. The percentage of glomeruli with crescents and global sclerosis was calculated as the percentage of the total number of glomeruli in a biopsy. Interstitial fibrosis and tubular atrophy are given as the percentage of the tubulointerstitial compartment affected. Deposition of IgA, IgG, IgM, C3, kappa and lambda light chains was scored semi-quantitatively by immunofluorescence staining.

*Meta-analysis*

*Literature search and study selection.* We performed a PubMed, EMBASE, and SCOPUS search to identify eligible articles. A forward search of the retrieved articles was performed and 'google scholar' was also assessed to screen for non-indexed publications. The last search was performed on December $12^{\text{th}}$, 2019. The search terms included: "ANCA vasculitis" OR "ANCA-associated vasculitis" OR "antineutrophil cytoplasmic antibody vasculitis" OR "AAV" AND complement. Publications were screened first by title, second by abstract, and finally by full-text, based on our eligibility criteria.

*Inclusion and exclusion criteria.* We included cross-sectional or longitudinal studies, which compared plasma levels of complement factors in patients with AAV and healthy controls. We excluded studies that have measured complement levels in the urine, other body fluids or biopsy specimens. The exclusion criteria also included review articles, case reports, and animal experiments.

*Data extraction and outcome.* Data extraction was carried out as recommended by the Cochrane handbook, and included authors, year of publication, study design, participants, demographic characteristics, and measurement of serum complements.

*Quality assessment.* This meta-analysis was conducted and reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement (Supplementary table S1). Risk of bias of individual studies at the outcome level was assessed by using the Newcastle-Ottawa scale. The scoring was performed independently by two researches (Lee JM and Shin JI) (Supplementary table S2).

*Statistical analysis and evaluation of heterogeneity and publication bias.* In the meta-analysis, the standard mean difference (SMD) method and corresponding 95% confidence intervals (CIs) were used to compare complement levels (mean±SD). Random effect models were used because of heterogeneity of the included studies. We assessed the heterogeneity of the studies by the Cochran Q test, and $P$ -value of $<0.1$ was considered significant. The inconsistency across the studies was also measured by $I^2$ metric, as a measure of the percentage of total variation across the studies because of the heterogeneity. $I^2$ values of $<25$, 25-75 and $>75\%$ considered to represent low, moderate, and high levels of heterogeneity, respectively. Publication bias of each article was estimated by inspecting funnel plot. All analyses were conducted using RevMan 5.3.

## Results

### Original study

*Demographic and clinical characteristics*

Fifty-nine patients with newly diagnosed or relapsing GPA (n=41) or MPA (n=18) were enrolled in this prospective study (Table 1). All patients presented with active vasculitis with a median BVAS of 16.5. Thirty-five patients had newly diagnosed AAV. Twenty-three of them were studied within 4-6 weeks after initiation of immunosuppressive therapy (up to 2-3 infusions of cyclophosphamide with high dose glucocorticoids) that was started before referral to our clinic, whereas 12 patients were not treated with any immunosuppressive agents at the time of testing. Twenty-four patients had relapsing AAV and continued maintenance immunosuppressive therapy at the time of testing. Six ANCA-negative patients had biopsy-proven localized GPA.

n 28 patients, testing was repeated in remission within 3 to 38 months (median 16 months) after the initiation of immunosuppressive treatment with rituximab (n=4), cyclophosphamide (n=20), methotrexate (n=3) or mycophenolate mofetil (n=1). Thirty-six healthy volunteers (9 males and 27 females, average age 55.7±12.4 years) comprised the control group.

*MAC, C5a, C3a, factors B and P (properdin) in patients with active AAV*

In patients with active AAV, the median concentrations of MAC, C5a, C3a, and factor B were higher than in the control group ($p<0.001$ for all comparisons; Table 2). Median properdin level was lower in patients with AAV. However, the difference between the two groups did not reach statistical significance. Concentrations of MAC, C5a, C3a, and factor B exceeded the upper reference levels in 50 (84.7%), 15 (25.4%), 49 (83.1%), and 8 (13.6%) patients respectively, whereas properdin level was decreased in 13 (22.0%) patients.

In 28 patients with AAV, we measured complement components levels both before and following remission induction. Achievement of remission was associated with a significant reduction in C3a from 21436.0 from to 11811.0 ng/ml ($p=0.005$), a decrease in C5a from 21.6 to 18.8 ng/ml ($p=0.035$), and factor B levels from 1804.0 to 1480.0 μg/ml ($p=0.045$). MAC and properdin levels did not change following remission induction therapy (Table 2).

Normalization of elevated complement component levels was achieved in 5 of 8 patients (62.5%) for C5a, in 11 of 25 patients (44.0%) for C3a, in 6 of 24 patients (25.0%) for MAC, and all 6 patients (100%) for factor B. Properdin levels in all 28 patients were normal both before and following remission induction treatment. Of note, in a few patients there was an increase in complement component levels following immunosuppressive treatment, including C3a in 2 patients, MAC in 3 patients, and factor B in 1 patient.

*Complement levels according to ANCA serotype, disease phenotype, new diagnosis or relapsing disease or treatment-naïve versus treated patients*

There was no difference in MAC, C5a, C3a, factor B, and properdin levels between PR3-ANCA positive and MPO-ANCA positive patients, between patients with GPA and MPA, between patients with newly diagnosed AAV or relapsing disease, and between treatment-naïve patients and those already receiving immunosuppression (Supplementary tables S3-6).

We also evaluated activation of the complement system in patients with 'non-severe', 'severe PR3-ANCA positive' and 'severe MPO-ANCA positive' AAV, representing the predominantly granulomatous, mixed granulomatous-vasculitic and predominantly vasculitic patterns of AAV [16]. Non-severe AAV (usually PR3-ANCA positive or sometimes ANCA-negative) was defined as granulomatosis (ENT-disease, lung nodules/masses, retroorbital tumor and/or pachymeningitis) without life/organ-threatening disease, whereas 'severe' vasculitis-related manifestations included glomerulonephritis, alveolar hemorrhage, mononeuritis multiplex, and scleritis. Median levels of all studied complement components were similar in these groups of patients (Supplementary table S7).

Furthermore, patients were distributed into two groups depending on the severity of disease that was defined as the presence of at least one major BVAS item and a total BVAS>6. Median BVAS scores in patients with severe and non-severe AAV were 15 (13;16) and 8 (6;9), respectively. Complement component levels did not differ between patients with severe and non-severe vasculitis (Supplementary table S8).

*Correlation between levels of various complement components and clinical and pathological parameters in patients with active AAV*

In 59 patients with active AAV, C5a levels had significant positive correlations with C3a (r=0.371, p=0.004), MAC (r=0.355, p=0.006), and factor B (r=0.281, p=0.031). However, there was no correlation between various complement components and BVAS, daily proteinuria, serum creatinine, C-reactive protein or erythrocyte sedimentation rate (ESR) (Supplementary table S9).

In 13 patients with kidney biopsies, factor B level correlated with C3 deposition in the glomeruli (r=0.648, p=0.023), whereas all other correlations between complement components and histological parameters did not reach statistical significance (Supplementary table S10).

**Meta-analysis**

*Study selection and characteristics*

A total of 1122 articles were identified using electronic and manual research (Supplementary figure 1). After reviewing titles and abstracts, 19 studies were selected for full-text reading. Of them, 14 were excluded (1 study was not available for the raw data) to finally include 4 eligible articles by December 12$^{th}$, 2019 [8-10, 17]. Among these, Gou et al. measured the levels of complement pathway factors twice at different time points, to which we refer as "Gou, sequential." In addition to this, we added the data from our current study. The respective characteristics of the included studies are described in detail in Supplementary table S11. The study quality assessed by the Newcastle-Ottawa scale scored 8 in three studies and 6 in one study (range, 1 (very poor) to 9 (very high), Supplementary table S2).

*Meta-analysis of complement levels*

The meta-analysis on MAC levels examined 178 patients with active AAV, 148 patients with AAV in remission, and 100 healthy controls. Plasma MAC levels were significantly higher in the active AAV group compared to patients in remission (SMD, 1.58, 95% confidence interval (CI), 0.22-2.93) and healthy controls (SMD, 1.50, 95% CI, 0.84-2.17) (Figure 1).

C5a levels were measured in 220 patients with active AAV, 192 patients with AAV in remission, and 124 healthy controls. Plasma C5a levels were significantly higher in the active AAV group compared to patients in remission (SMD, 0.80, 95% CI, 0.33-1.27) and healthy controls (SMD, 1.18, 95% CI, 0.56-1.80) (Figure

2). The remitted group showed higher plasma C5a levels when compared to controls (SMD, 1.19, 95% CI, 0.27-2.12).

The meta-analysis on C3a levels examined 223 patients with active AAV, 192 patients with AAV in remission, and 124 healthy controls. Plasma C3a levels had a marginal tendency to be higher in the active AAV group compared to patients in remission (SMD, 1.35, 95% CI, 0.20-2.51) and healthy controls (SMD, 1.87, 95% CI, 0.08-3.66), and also in the remitted AAV groups compared to healthy controls (SMD, 0.73, 95% CI, 0.04-1.41). Significance of C3a regulation was in general borderline (Figure 3).

Properdin levels were examined and compared in 158 active patients, 128 remitted patients, and 97 healthy controls, but did not show difference in any of the comparisons (Supplementary figure 2).

Plasma C4d levels were measured in 98 active AAV patients, 90 remitted AAV patients, and 58 healthy controls. The levels were higher in the active AAV group compared to healthy controls (SMD, 1.64, 95% CI, 0.34-2.94), and also elevated in AAV patients in remission compared to controls (SMD, 1.11, 95% CI, 0.31-1.92). C4d levels were not different between AAV patients in different disease activity states (Supplementary figure 3).

Plasma factor B levels were examined in 125 patients with active AAV, 82 patients with AAV in remission, and 75 healthy controls. The levels were higher in the active AAV group compared to controls (SMD, 0.83, 95% CI, 0.53-1.12), and also elevated in AAV patients in remission compared to controls (SMD, 0.87, 95% CI, 0.39-1.34) (Figure 4). Plasma Bb levels were investigated in 119 patients with active AAV, 120 patients with AAV in remission and 65 healthy controls. None of the comparisons significantly differed (Supplementary figure 4).

*Assessment of heterogeneity and publication bias*

We assessed statistical heterogeneity between the included studies. Since the $I^2$ test showed a value >50%, indicating substantial heterogeneity, we used random effect models for meta-analyses. The funnel plot showed near symmetry (Supplementary figure 5).

## Discussion

All three complement activation pathways converge at the generation of C3 convertase that cleaves C3 into anaphylatoxin C3a and opsonin C3b [18]. The association of C3b with the C3 convertase results in the formation of a C5 convertase cleaving C5 into anaphylatoxins C5a and C5b. This cleavage triggers the terminal complement cascade leading to the assembly of MAC (C5b-9). In our and previous studies, the terminal pathway components levels, that is C5a and MAC, were elevated in patients with active AAV compared to healthy controls. This finding indicates that complement activation occurs in the course of systemic vasculitis. In our patients, effective remission induction treatment was associated with a decrease in C5a levels, whereas MAC levels did not change. Nevertheless, the meta-analysis showed that both C5a and MAC levels in patients with remission of AAV were lower compared to patients with active disease. Remission induction therapy also resulted in a decrease in C3a levels. Activation of complement in AAV occurred irrespective of ANCA-serotype, severity of disease or previous immunosuppression, since we detected no difference between C5a and MAC levels in the groups of patients with MPO-ANCA or PR3-ANCA vasculitis, active MPA or GPA, severe or non-severe vasculitis, newly diagnosed or relapsing disease, predominant granulomatous or vasculitic or mixed disease, immunosuppressive-naïve or previously immunosuppressed patients. C5a and MAC levels did not correlate with BVAS, proteinuria, serum creatinine or laboratory markers of inflammation and were increased only in a proportion of patients with active AAV. Of note, C5a concentrations exceeded the upper reference level only in a quarter of patients who showed clinical signs of active AAV. Therefore, analysis of both complement components alone does not allow for distinction between active disease and remission.

We also measured factor B and properdin levels that are components of the alternative complement pathways. Factor B can be cleaved by factor D into Ba and Bb and is necessary for the formation of the alternative pathway C3 convertase (C3bBb), whereas properdin increases the half-life of the convertase activity and

promotes constant cleavage of C3 into C3a and C3b. Median factor B levels were elevated in patients with active AAV compared to healthy controls and decreased after remission. However, we detected no difference between median properdin levels in AAV patents and healthy volunteers. These data were confirmed by our meta-analysis.

Deposition of Bb in glomeruli of patients with AAV correlated with the proportion of crescents, the extent of interstitial infiltrates, interstitial fibrosis and tubular atrophy, and inversely with the proportion of normal glomeruli [19]. Moreover, plasma Bb concentrations correlated with common pathway components levels (C3a, C5a, and MAC), clinical and laboratory signs of vasculitis activity (BVAS, erythrocyte sedimentation rate (ESR)), and the proportion of total and cellular crescents in kidney biopsies [8]. These findings indicate that circulating Bb levels may reflect both systemic and renal disease activity of AAV. In another study that analyzed 187 renal biopsy samples from patients with AAV, properdin staining was associated with the proportion of cellular crescents, and the presence of properdin correlated with the level of proteinuria [5]. In our study, factor B or properdin levels did not correlate with proteinuria, serum creatinine, BVAS or laboratory markers of inflammation (ESR, C-reactive protein). In a small group of patients with kidney biopsies, factor B levels correlated with C3 deposition in the glomeruli, whereas all other correlations between complement components and histological parameters were not significant.

A crucial role of complement activation in AAV pathogenesis makes targeting complement components an attractive therapeutic strategy. Currently, two C5a inhibitors are in clinical development for AAV: avacopan, an oral C5a receptor (C5aR) inhibitor, and IFX-1, a monoclonal antibody to C5a [20]. Efficacy, safety and steroid-sparing effects of avacopan in patients with GPA/MPA were shown in two phase II trials, CLEAR and CLASSIC [20, 21], whereas IFX-1 has entered phase II development. The positive results of the phase III trial of avacopan, ADVOCATE, in AAV patients were recently announced, though not published yet. C5a also interacts with C5L2 receptors that compete with C5aR for binding of anaphylotoxins and, therefore, may have anti-inflammatory effects. Knockout of C5L2 in mice resulted in a more severe MPO-ANCA associated glomerulonephritis [4]. On the contrary, C5L2 was upregulated in glomeruli in patients with ANCA-associated glomerulonephritis [7]. These contradictory experimental and clinical data suggest that C5L2 may have different roles and functions in various systems, cells, tissues, organs, and/or species [22].

A major limitation of our study is the relatively small sample of patients, particularly studied sequentially both prior to and after achievement of remission. However, our findings are in accordance with previous studies and were reinforced by the meta-analysis of all available data on activation of the complement system in AAV.

In summary, our findings and the results of the meta-analysis provided additional evidence for activation of the complement system via the alternative pathway in AAV. Signs of complement activation were found only in a proportion of patients with active AAV, whereas achievement of clinical remission following immunosuppressive therapy did not always lead to normalization of various complement component levels. Therefore, we cannot conclude that any plasma complement component alone may be useful as a biomarker of the disease activity or a guide for treatment decisions. However, studies of the complement system in AAV and other autoimmune diseases pave the way to new drugs development and can turn the dream of steroid-free regimens into a reality. In addition, investigations of key signaling pathways and molecules (i.e., macrophage migration inhibitory factor, sphingosine-1-phosphate, high mobility group box 1) of C5a-mediated neutrophil priming and activation by ANCA may provide new insights into the complex AAV pathogenesis.

## Disclosure

The authors declare no conflicts of interest.

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**Tables**

**Table 1** . Demography and clinical features of 59 patients with ANCA-associated vasculitis.

| Parameters |
|---|
| Females, n (%) Average age (mean±SD), years Diagnosis, n (%) GPA MPA Newly diagnosed AAV, n (%) Visceral disease, |

Abbreviations: GPA – granulomatosis with polyangiitis, MPA – microscopic polyangiitis, PR3 – proteinase 3, MPO – myeloperoxidase, ESR – erythrocyte sedimentation, rate. *Median dose of prednisone was 12 (13; 22) mg. **Immunosuppressive medications included azathioprine (8), methotrexate (2) and mycophenolate mofetil (2).

**Table 2.** Complement component levels in patients with serial measurements (active and remission), and during disease activity compared to healthy controls.

| | Active AAV vs. controls | Active AAV vs. controls | Active AAV vs. remission | Active AAV vs. |
|---|---|---|---|---|
| | Active AAV (n=59) | Control (n=36) | Active AAV (n=28) | Remission (n=28 |
| C5a, ng/ml | 22.9 (14.4; 33.0) | 3.0 (0.4; 6.7)*** | 21.6 (21.7; 46.0) | 18.8 (14.6; 25.7) |
| C3a, ng/ml | 21436.0 (11395.0; 21436.0) | 1224.5 (798.5; 1947.7)*** | 21436.0 (17102.0; 20971.3) | 11811.0 (11003.0 |
| MAC, mAU/ml | 24646.0 (15342.0; 46681.0) | 3305.5 (2780.2; 3777.5)*** | 29286.0 (23448.3; 43048.5) | 20057.4 (19709.3 |
| Factor B, μg/ml | 1586.0 (1175.0; 2145.0) | 1013.5 (770.7; 1548.5)*** | 1804.0 (1514.6; 2438.0) | 1480.0 (1124.3; 1 |
| Properdin, μg/ml | 402.0 (360.0; 447.0) | 416.0 (400.2; 437.0) | 388.0 (372.0; 417.2) | 402.0 (381.9; 423 |

Note: *p<0.05, ***p<0.001. Changes in complement component levels were studied in 28 patients who have undergone repeated testing following remission induction therapy. Using data from the control group, the upper reference levels of the complement components were defined as following: MAC – 11271.1 mAU/ml, C5a – 33.1 ng/ml (three outliers were excluded), C3a – 8955.3 ng/ml, factor B – 2864.1 μg/ml. The lower reference level of properdin was defined as a cut-off of 357.8 μg/ml. Abbreviations: AAV (ANCA-associated vasculitis), MAC (membrane attack complex).

**Figures**

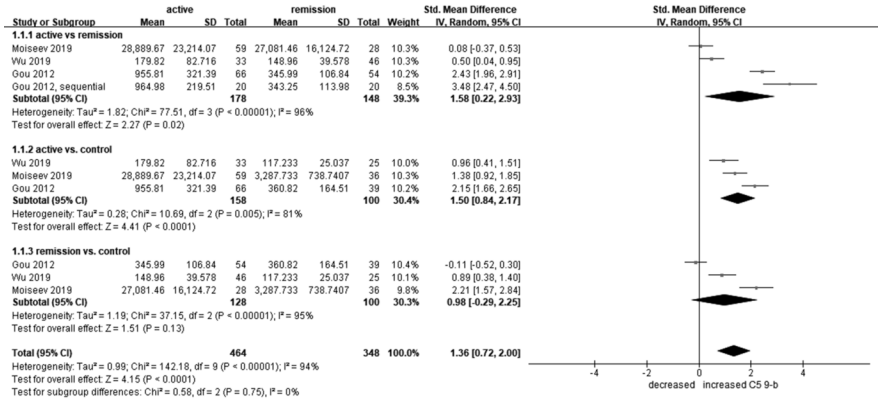



**Figure 1.** Forest plot of random effects meta-analysis of MAC levels.

1.1.1. AAV patients in active state vs. in remission; 1.1.2. active AAV patients vs. healthy controls; 1.1.3. AAV patients in remission vs. healthy controls. Squares are proportional to study weight. AAV - ANCA-associated vasculitis.

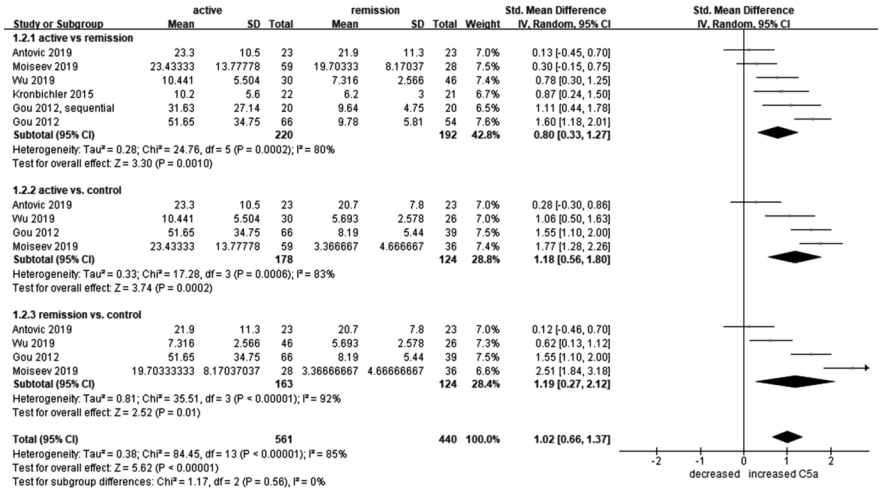



**Figure 2.** Forest plot of random effects meta-analysis of C5a levels.

1.2.1. AAV patients in active state vs. in remission; 1.2.2. active AAV patients vs. healthy controls; 1.2.3. AAV patients in remission vs. healthy controls. Squares are proportional to study weight. AAV - ANCA-associated vasculitis.

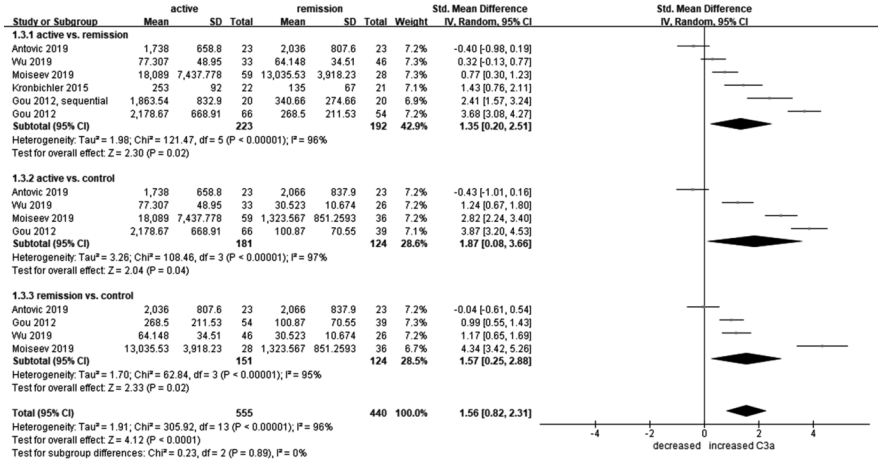
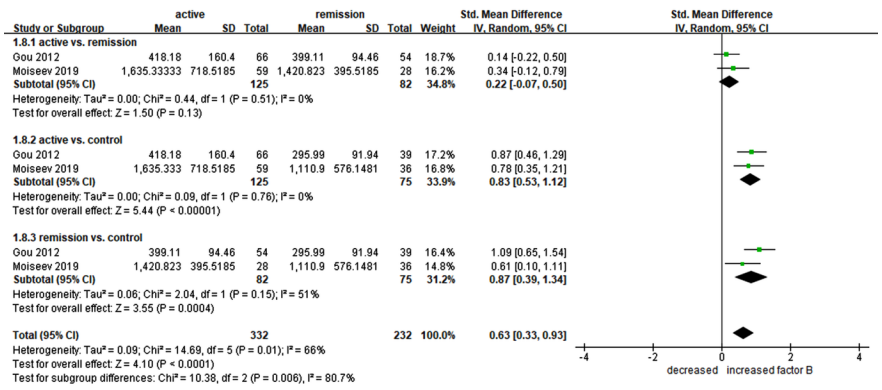



**Figure 3.** Forest plot of random effects meta-analysis of C3a levels.




1.3.1. AAV patients in active state vs. in remission; 1.3.2. active AAV patients vs. healthy controls; 1.3.3. AAV patients in remission vs. healthy controls. Squares are proportional to study weight. AAV - ANCA-associated vasculitis.

**Figure 4.** Forest plot of random effects meta-analysis of factor B levels.

1.8.1. AAV patients in active state vs. in remission; 1.8.2. active AAV patients vs. healthy controls; 1.8.3. AAV patients in remission vs. healthy controls. Squares are proportional to study weight. AAV - ANCA-associated vasculitis.