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AUTHOR'S SUMMARY

VASCULAR EFFECTS OF BIOLOGIC AND TARGETED SYNTHETIC THERAPY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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The dissertation comprises **179 standard pages** and is illustrated with **64 tables** and **28 figures**. The bibliography includes **521 references**. The dissertation was reviewed and approved for public defense at a meeting of the Departmental Council of the **Department of Propaedeutics of Internal Medicine**, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna.

CONTENTS

List of Abbreviations

AC – Arterial Compliance
ACPA – Anti-Citrullinated Protein Antibodies
ACR – American college of rheumatology
ADMA – Asymmetric Dimethylarginine
AIx – Augmentation Index
ANOVA – Analysis of Variance
APO – Apolipoprotein
ASA – Acetylsalicylic Acid
AZA – Azathioprine
BMI – Body Mass Index
BP – Blood Pressure
CAD – Coronary Artery Disease
CAVI – Cardio-Ankle Vascular Index
CCP – Cyclic Citrullinated Peptide antibodies
CDAI – Clinical Disease Activity Index
CI – Confidence Interval
CIMT – Carotid Intima-Media Thickness
CRP – C-Reactive Protein
DAS – Disease Activity Score
DAS28 – Disease Activity Score 28
DDAH – Dimethylarginine Dimethylaminohydrolase
DMARD – Disease-Modifying Anti-Rheumatic Drug
EDTA – Ethylenediaminetetraacetic Acid
EMA – European Medicines Agency
EMP – Endothelial microparticles
ESR – Erythrocyte Sedimentation
EULAR – European Alliance of Associations for Rheumatology
FDA – Food and Drug Administration
FMD – Flow-Mediated Dilatation
FRS – Framingham Risk Score
HAQ – Health Assessment Questionnaire
HCQ – Hydroxychloroquine
HDL – High-Density Lipoprotein
HR – Hazard Ratio
hsCRP – High-sensitivity C-reactive protein
IL – Interleukin
JAK – Janus Kinase
KDR – Kinase Insert Domain Receptor

LDF – Laser Doppler Flowmetry
LDI – Laser Doppler Imaging
LDL – Low-Density Lipoprotein
MACE – Major Adverse Cardiovascular Events
MCP – Metacarpophalangeal joint
MCV – Modified Citrullinated Vimentin
MHC – Major histocompatibility complex
MMP – Matrix Metalloproteinases
MPO – Myeloperoxidase
MTX – Methotrexate
NET – Neutrophil Extracellular Trap
NMMA – NG-Monomethyl-L-Arginine
NO – Nitric Oxide
NOS – Nitric oxide synthase
PAD – Peptidyl Arginine Deiminase
PAT – Peripheral Arterial Tonometry
PGA – Patient Global Assessment
PIP – Proximal Interphalangeal joint
PON1 – Paraoxonase 1
PORCH – Post occlusive reactive hyperemia
PTPN22 – Protein Tyrosine Phosphatase Non-receptor type 22
PTX3 – Pentraxin
PWV – Pulse Wave Velocity
RA – Rheumatoid Arthritis
RANK – Receptor Activator of Nuclear Factor κB
RANKL – Receptor Activator of Nuclear Factor κB Ligand
RF – Rheumatoid Factor
RTX – Rituximab
SAA – Serum amyloid A
SD – Standard Deviation
SDAI – Simplified Disease Activity Index
SDMA – Symmetric Dimethylarginine
SE – Standard Error
SJC – Swollen Joint Count
SMD – Standardized Mean Difference
SMR – Standardized mortality ratio
STAT – Signal Transducer and Activator of Transcription
TC – Total Cholesterol
TG – Triglycerides

TGFB1 – Transforming Growth Factor Beta 1
TJC – Tender Joint Count
TNF – Tumor Necrosis Facto
TSP-1 – Thrombospondin-1
TYK2 – Tyrosine Kinase 2
UPA – Upadacitinib
VAS – Visual analogue scale
VCAM – Vascular Cell Adhesion Molecule
VEGF – Vascular Endothelial Growth Factor
VOP – Venous Occlusion Plethysmography
WMD – Weighted Mean Difference

I. INTRODUCTION

Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory joint disease. It is characterized by a complex etiopathogenesis resulting from the interaction between genetic predisposition and environmental factors, among which cigarette smoking is the most strongly associated risk factor. Despite the availability of modern classification criteria, early and accurate diagnosis remains challenging due to the marked clinical heterogeneity of the disease. The pathophysiology of RA involves complex immunological mechanisms leading to loss of immune tolerance and the formation of autoantibodies, primarily anti-citrullinated protein antibodies (ACPA) and rheumatoid factor (RF). These autoantibodies, in combination with the activation of innate and adaptive immunity, contribute to the development of persistent synovial inflammation, tissue remodeling, and progressive joint destruction. Immunopathological differences between seropositive and seronegative RA further account for the variability in clinical presentation and therapeutic response. As a systemic disease, RA affects multiple organs and systems and may manifest with a wide spectrum of extra-articular involvement, including pulmonary, cardiovascular, hematological, and neurological complications. These manifestations lead to significant impairment of quality of life, disability, and increased mortality. The introduction of the treat-to-target strategy and the early use of conventional, biological, and targeted synthetic disease-modifying antirheumatic drugs (DMARDs) have substantially improved disease control and delayed structural progression. Nevertheless, residual cardiovascular risk and late complications remain a major clinical challenge.

Rheumatoid arthritis is associated with an increased and frequently underestimated cardiovascular risk that cannot be explained solely by traditional risk factors. Early vascular injury—endothelial dysfunction and increased arterial stiffness—represents key pathophysiological steps in the development of atherosclerosis in this population. Endothelium-mediated vasodilation, serological markers such as asymmetric dimethylarginine (ADMA), and instrumental indices such as pulse wave velocity (PWV) provide opportunities for the early detection of subclinical vascular damage and for a more accurate assessment of cardiovascular risk. Data from the literature indicate that biological and targeted synthetic DMARDs may partially modify these early vascular changes, including endothelial function, arterial stiffness, carotid intima–media thickness, and serological markers such as ADMA. The most consistent evidence supports the beneficial vascular effects of tumor necrosis factor (TNF) inhibitors, while interleukin-6 (IL-6) inhibitors also demonstrate a favorable vascular

profile. In contrast, the cardiovascular safety of Janus kinase (JAK) inhibitors remains a subject of ongoing debate, particularly in the context of the multifactorial and substantially increased cardiovascular risk in RA. Optimization of therapeutic strategies therefore requires an individualized approach that takes into account not only the anti-inflammatory efficacy of a given medication but also its impact on cardiovascular outcomes.

The need for further comparative studies remains critical for a better understanding of the vascular effects of various targeted therapies and for the precise management of cardiovascular risk in patients with rheumatoid arthritis. The present dissertation addresses the relationship between inflammatory activity and parameters of arterial stiffness in patients treated with different biological therapies. The object of the study is patients with rheumatoid arthritis, while the subject is the effect of anti-inflammatory treatment on markers of vascular function and the risk of atherosclerotic changes. The aim is to evaluate the effects of TNF-α inhibitors and JAK inhibitors on arterial elasticity and the associated biochemical and clinical parameters. To achieve these objectives, clinical, laboratory, and instrumental methods were applied, including the assessment of arterial stiffness indices (PWV, β-stiffness index, AI, AC, EP), analysis of biochemical markers (lipid profile, CRP, ADMA), and statistical models for comparative, correlation, and regression analyses.

II. AIM AND OBJECTIVES

1. Aim

The aim of this dissertation is to compare the parameters of ultrasound-assessed arterial stiffness, serum levels of asymmetric dimethylarginine (ADMA), and lipid profile parameters among three groups: patients with rheumatoid arthritis treated with the TNF inhibitor adalimumab, patients treated with the JAK inhibitor upadacitinib, and a control group of healthy individuals.

2. Objectives

1. To assess ultrasound-derived indices of arterial stiffness as markers of early vascular damage in patients with rheumatoid arthritis and to compare them among the three study groups: TNF inhibitor therapy, upadacitinib therapy, and healthy controls.
2. To investigate serum ADMA levels as a biomarker of endothelial dysfunction and to compare them between the therapeutic groups and the control group.

3. To analyze the impact of lipid profile parameters on vascular indices and to evaluate the relationship between lipid levels, treatment modality, and early vascular changes.
4. To assess the role of disease activity and the applied composite disease activity indices (DAS28-ESR, DAS28-CRP, CDAI) on markers of arterial stiffness and ADMA, including the influence of remission, low, moderate, and high disease activity.
5. To determine cardiovascular risk using the Framingham Risk Score and to identify independent factors associated with early vascular damage in patients with rheumatoid arthritis.

III. MATERIALS AND METHODS

1. Study Design

A cross-sectional observational study was conducted. The study population consisted of 79 patients with rheumatoid arthritis and 30 healthy volunteers. After providing written informed consent for participation, all patients were interviewed, completed self-assessment scales for disease activity and pain, and underwent a comprehensive joint examination. Following medical history taking and physical examination, blood samples were collected in three tubes: one EDTA tube for complete blood count and erythrocyte sedimentation rate (ESR), and two serum tubes. The first serum sample was used to measure C-reactive protein (CRP), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lipid profile parameters (total cholesterol, triglycerides, HDL, LDL). The second serum sample was separated and stored frozen for subsequent measurement of asymmetric dimethylarginine (ADMA). Based on the laboratory results, clinical examination, and self-assessment scales, disease activity indices were calculated, including DAS28-ESR, DAS28-CRP, and CDAI, as well as the Framingham Risk Score for cardiovascular risk assessment. On the same day, arterial blood pressure was measured, carotid artery ultrasound was performed, and arterial stiffness parameters were assessed. Patients were further divided into two therapeutic groups: patients treated with TNF inhibitors and patients treated with the JAK inhibitor upadacitinib. Prior to initiation of the study, ethical approval was obtained from the Ethics Committee of the Medical University of Varna.

2. Patients and Controls

Over a two-year period, a total of 41 patients treated with TNF inhibitors, 38 patients treated with upadacitinib, and 30 healthy controls were included in the study. Recruitment of the therapeutic groups was carried out through the outpatient registry of patients with inflammatory joint diseases receiving biological and targeted synthetic disease-modifying antirheumatic drugs at the Rheumatology Clinic of St. Marina University Hospital. This registry follows more than 2,000 patients with inflammatory joint diseases who are treated with anti-cytokine therapies and are monitored at six-month intervals. According to Bulgarian regulations, patients treated with biological or targeted synthetic DMARDs are required to have experienced failure of at least two conventional synthetic DMARDs prior to initiation of such therapies. Healthy controls were recruited from the staff of St. Marina University Hospital and the Medical University of Varna, as well as from healthy volunteers and patients admitted to the rheumatology clinic for degenerative joint diseases, without inflammatory joint diseases or systemic connective tissue disorders.

3. **Inclusion Criteria**

For the purposes of the present study, patients diagnosed with rheumatoid arthritis according to the ACR/EULAR criteria for early RA or the modified New York criteria for established RA were selected and evaluated. To be eligible for inclusion, patients had to have been treated with a TNF inhibitor or with the JAK inhibitor upadacitinib for at least 6 months. All participants were required to be over 18 years of age, fully oriented to time, place, and person, and capable of reading, understanding, and personally signing the informed consent form for study participation.

The control group was selected among individuals over 18 years of age who were also capable of reading, understanding, and personally signing the informed consent form and who met none of the exclusion criteria.

4. Exclusion Criteria

Patients meeting one or more of the following criteria were excluded from the study:

1. Known coronary atherosclerosis documented by exercise testing, coronary angiography, or other diagnostic methods.
2. Known heart failure.
3. Known valvular heart disease.
4. Chronic atrial fibrillation.

5. History of ischemic stroke, known cerebrovascular disease, or vertebrobasilar insufficiency.
6. Known peripheral arterial disease.
7. Chronic kidney disease stage IIIA or more advanced.
8. Type 1 or type 2 diabetes mellitus.
9. Treatment with systemic glucocorticoids at a dose >10 mg/day of prednisolone equivalent.
10. Known systemic connective tissue disease overlapping with RA (e.g., systemic lupus erythematosus, Sjögren's syndrome, etc.).
11. Known malignancy that was active or diagnosed within 5 years prior to study inclusion.
12. Disorientation to time, place, or person.
13. Age under 18 years.
14. Illiteracy or inability to read, understand, and personally sign the informed consent form.

The same exclusion criteria applied to the control group. In addition, the presence of any other type of inflammatory joint disease (e.g., spondyloarthritis, psoriatic arthritis, etc.) was considered an exclusion criterion for controls.

5. Clinical Methods

5.1. Medical History

The following information was collected from all study participants during medical history assessment:

1. Sex, age, height, and body weigh
2. Presence of arterial hypertension
3. Duration of rheumatoid arthritis
4. Current treatment regimen
5. Previous biological therapies
6. Smoking status

5.2 Clinical Examination

5.2.1 Blood Pressure Measurement

Blood pressure measurements were performed at rest in a controlled environment with an ambient temperature of 22–24°C, following at least a 5-minute adaptation period in the seated position. All measurements were conducted in accordance with the European Society of Hypertension (ESH) recommendations for good clinical practice in ambulatory blood pressure measurement [Parati G, et al. J Hypertens. 2014;32:1359–66]. Blood pressure was measured on the right brachial artery using a manual oscillometric device. An appropriately sized cuff was applied (width ≈40% and length ≈80% of the upper arm circumference) at heart level. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded. For each participant, at least two consecutive measurements were obtained at 1–2-minute intervals. If the difference between the two values exceeded 5 mmHg, a third measurement was performed, and the mean value of the last two readings was used for the statistical analysis.

5.2.2 Clinical Assessment of Joint Involvement and Calculation of Disease Activity Indices

The clinical assessment of joint involvement included the evaluation of the number of **tender joints (TJC – tender joint count)** and **swollen joints (SJC – swollen joint count)** in each patient. A **28-joint assessment protocol** was used, which includes the following joints bilaterally: shoulders, elbows, wrists, metacarpophalangeal joints (MCP I–V), proximal interphalangeal joints (PIP I–V), and knees. This method is integrated into composite disease activity indices and is characterized by easier implementation and lower inter-examiner variability compared with the 66/68 joint count methodology [Grünke M, et al. J Rheumatol. 2012;39:1334–40].

Three scales were used for the assessment of disease activity by both the patient and the physician:

5.2.3 Patient Global Assessment of Disease Activity (PGA)

This is a subjective assessment in which the patient evaluates their overall condition and the severity of the disease at the time of examination. The assessment is performed using a verbal scale ranging from 0 to 10, where 0 indicates complete absence of disease activity (full remission) and 10 corresponds to the highest possible disease activity (severe symptoms).This measure reflects the patient’s personal perception of symptom intensity, including pain, stiffness, and functional limitation [Felson DT, et al. Arthritis Rheum. 1993;36:729–40].

5.2.4 Physician Global Assessment of Disease Activity

This scale reflects the clinical judgment of the examining physician regarding the degree of disease activity at the time of assessment. The evaluation is based on physical examination, the number of tender and swollen joints, medical history, and, when available, additional data (e.g., laboratory findings). The scale also ranges from 0 to 10, where 0 indicates no disease activity and 10 represents extremely high disease activity. This assessment allows for the objectification of the physician's subjective clinical observations regarding disease activity [Harrington JT. J Rheumatol. 2009;36:925–9].

5.2.5 Visual Analogue Scale (VAS, 0–100 mm)

The Visual Analogue Scale (VAS) consists of a horizontal 100-mm line on which the patient marks the current intensity of their symptoms. The endpoints of the line correspond to: 0 mm – no complaints (completely controlled disease) and 100 mm – the most severe imaginable complaints (extremely high disease activity). The VAS is easy to administer, widely used in both clinical practice and research, and has broad application in rheumatology for the subjective assessment of parameters such as pain, fatigue, disease activity, and others [Levy O, et al. Isr Med Assoc J. 2015;17:691–6].

5.3 Disease Activity Indices

For the assessment of disease activity, three validated indices were applied: DAS28-ESR, DAS28-CRP, and CDAI.

5.3.1. DAS28-ESR and DAS28-CRP

To objectively assess disease activity in patients with rheumatoid arthritis, two widely established composite indices were used in the present study: DAS28-ESR and DAS28-CRP. Both indices are based on the same clinical parameters and differ only in the acute-phase reactant used: erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), respectively.

Parameters included in the calculation:

- Tender joint count (TJC28) – from a total of 28 joints
- Swollen joint count (SJC28) – from the same 28 joints
- Patient global assessment of overall health status (patient VAS, 0–100 mm)
- Laboratory marker of inflammation – ESR (for DAS28-ESR) or CRP (for DAS28-CRP)

The scores were calculated using the following formulas:

- DAS28-ESR = 0.56 × TJC28 + 0.28 × SJC28 + 0.70 × ln(ESR) + 0.014 × VAS
- DAS28-CRP = 0.56 × TJC28 + 0.28 × SJC28 + 0.36 × ln(CRP + 1) + 0.014 × VAS + 0.96

Based on the obtained values, patients were categorized into four disease activity states:

- ≤ 2.6 – Remission
- 2.6–3.2 – Low disease activity
- > 3.2–5.1 – Moderate disease activity
- ≥ 5.2 – High disease activity

The DAS28-ESR and DAS28-CRP indices are routinely used in both clinical practice and clinical trials. They allow for longitudinal monitoring of disease activity and enable the implementation of the treat-to-target strategy in everyday clinical settings. It should be noted that the two composite indices are not interchangeable, as they may yield discordant information [Shivacheva TK. Folia Med (Plovdiv). 2020;62:46–51].

All calculations in the present study were performed using validated electronic calculators, available through specialized platforms (e.g., the EULAR DAS28 calculator).

5.3.2. Clinical Disease Activity Index (CDAI)

The Clinical Disease Activity Index (CDAI) is a validated composite measure used for the quantitative assessment of disease activity in patients with rheumatoid arthritis. Its main advantage lies in the fact that it relies exclusively on clinical parameters, without the need for laboratory tests, which makes it particularly suitable for routine clinical practice and outpatient settings.

Components of CDAI:

1. Tender joint count (TJC28) – out of 28 assessed joints
2. Swollen joint count (SJC28) – from the same 28 joints
3. Physician global assessment of disease activity (0–10 scale)
4. Patient global assessment of disease activity (0–10 scale)

The index is calculated using the following formula:
CDAI = TJC28 + SJC28 + PGA + Physician Global

The final score represents the sum of these four parameters, with a minimum value of 0 (no disease activity) and a theoretical maximum of 76. Based on the total score, CDAI defines four disease activity categories:

- Remission: ≤ 2.8
- Low activity: > 2.8–10
- Moderate activity: > 10–22
- High activity: > 22

CDAI is an especially valuable tool in situations where laboratory markers (e.g., CRP, ESR) are unavailable, and its use is further facilitated by the absence of complex formulas. Importantly, the omission of inflammatory biomarkers may be advantageous in patients treated with cytokine-targeted therapies [Koh JH et al. Ther Adv Musculoskelet Dis. 2022;14].

5.4. Assessment of Cardiovascular Risk Using the Framingham Risk Score (FRS)

The Framingham Risk Score (FRS) is a widely used and well-established epidemiological tool for estimating an individual's 10-year risk of developing cardiovascular disease (CVD) [Wilson PWF et al., *Circulation*. 1998;97:1837–1847]. It was developed based on data derived from the long-term Framingham Heart Study [Sytkowski PA et al., *N Engl J Med*. 1990;322:1635–1641].

FRS incorporates the following variables:

- Sex (male/female)
- Age
- Total serum cholesterol
- High-density lipoprotein (HDL) cholesterol
- Systolic blood pressure (mmHg), with documentation of whether antihypertensive treatment is being used
- Smoking status (yes/no)
- Diabetes mellitus (yes/no)

Calculation and Interpretation

Risk estimation is performed using sex-specific equations:

- For men:
  LMen = β × ln(Age) + β × ln(Total cholesterol) + β × ln(HDL cholesterol) + β × ln(Systolic BP) + β × Treated for hypertension + β × Smoker + β × ln(Age) × ln(Total cholesterol) + β × ln(Age) × Smoker + β × ln(Age) × ln(Age) − 172.300168
- For women:
  LWomen = β × ln(Age) + β × ln(Total cholesterol) + β × ln(HDL cholesterol) + β × ln(Systolic BP) + β × Treated for hypertension + β × Smoker + β × ln(Age) × ln(Total cholesterol) + β × ln(Age) × Smoker − 146.5933061

The derived value is subsequently transformed into a percentage estimate of the 10-year risk of a cardiovascular event, including myocardial infarction, coronary death, stroke, and other major cardiovascular outcomes. Based on the calculated score, individuals are categorized into three risk groups:

- Low risk: FRS < 10%
- Moderate risk: FRS 10–20%
- High risk: FRS > 20%

In patients with rheumatoid arthritis, cardiovascular risk is increased due to chronic systemic inflammation. Therefore, according to the recommendations of the European League Against Rheumatism (EULAR), the calculated FRS should be multiplied by a factor of 1.5 to account for this additional inflammation-related risk [Agca R et al., *Ann Rheum Dis*. 2017;76:17–28].

In the present study, FRS was used for the quantitative assessment of baseline cardiovascular risk in the enrolled patients, as well as for the analysis of potential correlations between cardiovascular risk, markers of vascular dysfunction, and rheumatoid arthritis disease activity.

6. Serological Methods

6.1. Measurement of Erythrocyte Sedimentation Rate (ESR)

The erythrocyte sedimentation rate (ESR) is a nonspecific laboratory marker that reflects the presence and degree of systemic inflammatory activity. Elevated ESR values are commonly

observed in autoimmune diseases, including rheumatoid arthritis (RA), and are routinely used for assessing disease activity and monitoring therapeutic response.

Measurement Method

In the present study, ESR was determined using an automated capillary photometry method [Jou JM et al., *Int J Lab Hematol*. 2011;33:125–132]. This method is a modern alternative to the classical Westergren technique and is characterized by shorter analysis time, improved precision, and full automation.

- Principle of the method:
  A blood sample anticoagulated with sodium citrate is introduced into a specialized capillary system. Changes in optical density along the capillary are measured photometrically at fixed intervals (typically 20–30 minutes). The sedimentation rate is calculated automatically and extrapolated to the value corresponding to 60 minutes in the Westergren method.
- Instrument used: *Sysmex 1000XN*
- Reference values:
  For the purposes of this study, the normal reference interval for ESR was defined as 2–37 mm/h, according to the laboratory standards of University Hospital “St. Marina.”

Clinical Significance

ESR is incorporated into the calculation of the DAS28-ESR disease activity index, one of the primary composite measures used in the assessment of RA. The application of the capillary photometry method in routine laboratory practice provides a rapid, reliable, and reproducible evaluation of inflammatory status.

6.2. Measurement of C-Reactive Protein (CRP)

C-reactive protein (CRP) is an acute-phase plasma protein synthesized primarily by hepatocytes in response to inflammatory stimuli, with interleukin-6 (IL-6) being its strongest inducer [Zhou HH et al., *Front Immunol*. 2024;15]. CRP is routinely used in clinical practice for the assessment of systemic inflammation and is widely applied in monitoring disease activity in inflammatory rheumatic disorders, including rheumatoid arthritis (RA).

In the present study, serum CRP concentration was determined using a latex-enhanced immunoturbidimetric assay. Analyses were performed on an automated biochemical analyzer (ADVIA 1800 + IMMULITE 2000i). This method is based on the agglutination reaction between latex microspheres coated with monoclonal antibodies against human CRP and the CRP antigen present in the sample.

- Principle of the method:
  The formation of immune complexes between anti-CRP antibodies and CRP leads to an increase in sample turbidity. The intensity of turbidity is directly proportional to the CRP concentration and is measured photometrically at a specific wavelength.
- Reference values: 0–5.0 mg/L
  Values above the upper reference limit are considered indicative of an active inflammatory process.
- Advantages of the method:
  - High sensitivity and specificity
  - Short analysis time
  - Fully automated performance with minimal risk of operator-related error

Clinical Significance

CRP is a key biomarker of inflammatory activity in patients with RA and is incorporated into the calculation of the DAS28-CRP composite disease activity index, which is a well-established tool for objective disease assessment. Compared with the erythrocyte sedimentation rate (ESR), CRP responds more rapidly and dynamically to changes in clinical status, making it a preferred marker for monitoring therapeutic response and disease progression.

6.3. Assessment of the Lipid Profile

The lipid profile is a standard laboratory test panel used to evaluate key parameters related to lipid metabolism and the risk of atherosclerosis and cardiovascular disease. Measurement of serum lipids is particularly important in patients with rheumatoid arthritis (RA), in whom chronic inflammation and immunosuppressive therapies may affect lipid homeostasis [Yan J et al., *Front Immunol*. 2023;14].

Parameters included in the lipid profile:

1. Total Cholesterol (TC)
    - Reflects the total amount of cholesterol in the blood, including HDL, LDL, and other lipoprotein fractions.
    - Reference range: <5.2 mmol/L (optimal).
2. Triglycerides (TG)
    - The main storage form of lipids in the body; elevated levels are associated with increased cardiovascular risk.
    - Reference range: <1.7 mmol/L.
3. High-Density Lipoprotein Cholesterol (HDL-C)
    - Known as the "good" cholesterol due to its role in reverse cholesterol transport and vascular protection.
    - Reference range: >1.0 mmol/L in men; >1.3 mmol/L in women.
4. Low-Density Lipoprotein Cholesterol (LDL-C)
    - Referred to as the "bad" cholesterol; elevated levels are strongly linked to atherosclerosis.
    - Reference range: <3.0 mmol/L (for individuals with low to moderate cardiovascular risk).

Methodology

Analyses were performed on venous blood samples collected after a minimum of 8–12 hours of fasting. All tests were carried out under standard clinical laboratory conditions.

Method of determination:

- Serum concentrations of TC, TG, and HDL-C were measured using enzymatic colorimetric assays on an automated biochemical analyzer (ADVIA 1800).
- LDL-C was calculated using the Friedewald formula (when TG < 4.5 mmol/L):

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - (2.2 \times \text{TG})$$

Clinical Significance

Monitoring the lipid profile in patients with RA is essential for comprehensive assessment of cardiovascular risk. Lipid parameters were used in this study to calculate the Framingham Risk Score (FRS). The results also served to evaluate how lipid metabolism is influenced across the three study groups:

- RA patients treated with upadacitinib,
- RA patients treated with TNF inhibitors, and
- Healthy controls.

6.4. Measurement of Asymmetric Dimethylarginine (ADMA)

The concentration of endogenous asymmetric dimethylarginine (ADMA) in serum and plasma was determined using a quantitative enzyme-linked immunosorbent assay (ELISA), employing the ADMA Fast ELISA kit (DLD Diagnostika GmbH, Germany). ADMA is an endogenous inhibitor of nitric oxide synthase (NOS); when present at pathologically elevated levels or administered intra-arterially, it suppresses vascular nitric oxide (NO) production and induces local vasoconstriction [Böger R. *Cardiovasc Res.* 2003;59:824–833].

Principle of the Method

The ADMA Fast ELISA is a competitive immunoassay in which the pre-acylated ADMA from the samples competes with N-acyl-ADMA immobilized on a microtiter plate for a limited amount of rabbit anti–N-acyl-ADMA antibodies. After washing to remove unbound components, detection is performed using a peroxidase-conjugated anti-rabbit IgG antibody and a TMB substrate reaction, measured at 450 nm. The signal intensity is inversely proportional to the ADMA concentration in the sample.

Calibration and Quality Control

- Six calibration standards (0, 0.2, 0.45, 0.7, 1.0, and 3.0 µmol/L) and two control sera with known ADMA concentrations were used.
- Optical densities were analyzed using four-parameter logistic (4PL) regression.
- Concentrations were reported in µmol/L; when needed, the following conversion factor was applied:
  1 µmol/L = 202 ng/mL.

Method Characteristics

- Limit of detection: 0.03 µmol/L
- Calibration range: 0.2–3.0 µmol/L
- Linear range: up to 1:6 dilution with >90% recovery
- Intra- and inter-assay variability: 4.3–9.6%
- Specificity: No significant cross-reactivity with structurally related analogues such as SDMA, NMMA, or arginine.

Additional Notes

This assay allows sensitive and specific quantification of ADMA with low inter-assay variability and includes quality controls to ensure methodological reliability. Two ELISA kits were used in total, each capable of processing 80 samples (160 samples overall). As a result, 51 samples were tested in duplicate, distributed equally across the three study groups; the mean value of the duplicate measurements was used for the final dataset.

7. Assessment of Arterial Stiffness Using Aloka ProSound Alpha 7

Arterial stiffness was assessed using the Aloka ProSound Alpha 7 Doppler ultrasound system, equipped with a high-frequency linear transducer (12 MHz) and dedicated software for the automatic calculation of vascular hemodynamic parameters [Vriz O et al., *SAGE Open Med.* 2013]. This technique enables non-invasive and highly precise evaluation of vascular elasticity and compliance through synchronized detection of the arterial pulse wave and real-time measurement of the vascular lumen.

The following standardized examination protocol was applied:

- All measurements were performed in the morning hours, after a 12-hour fasting period and following at least 10–15 minutes of physical and psychological rest, in a climate-controlled room with a temperature of approximately 22°C.
- Patients were examined in the supine position, with the arms relaxed, and were instructed to avoid any movement during the examination.
- Blood pressure was measured immediately prior to the ultrasound examination using the previously described standardized procedure.

- Electrodes for three-channel peripheral electrocardiographic (ECG) recording were applied to ensure synchronization with the cardiac cycle.
- Ultrasonographic assessment of the distal segment of the right common carotid artery was performed approximately 1 cm proximal to the carotid bifurcation. After vessel visualization, the two measurement lines were precisely positioned along the inner and outer arterial walls at the level of the intima.
- A minimum of three cardiac cycles is required for valid measurement; however, in the present study ten consecutive cardiac cycles were recorded, from which at least five optimal cycles were selected to ensure greater measurement accuracy.

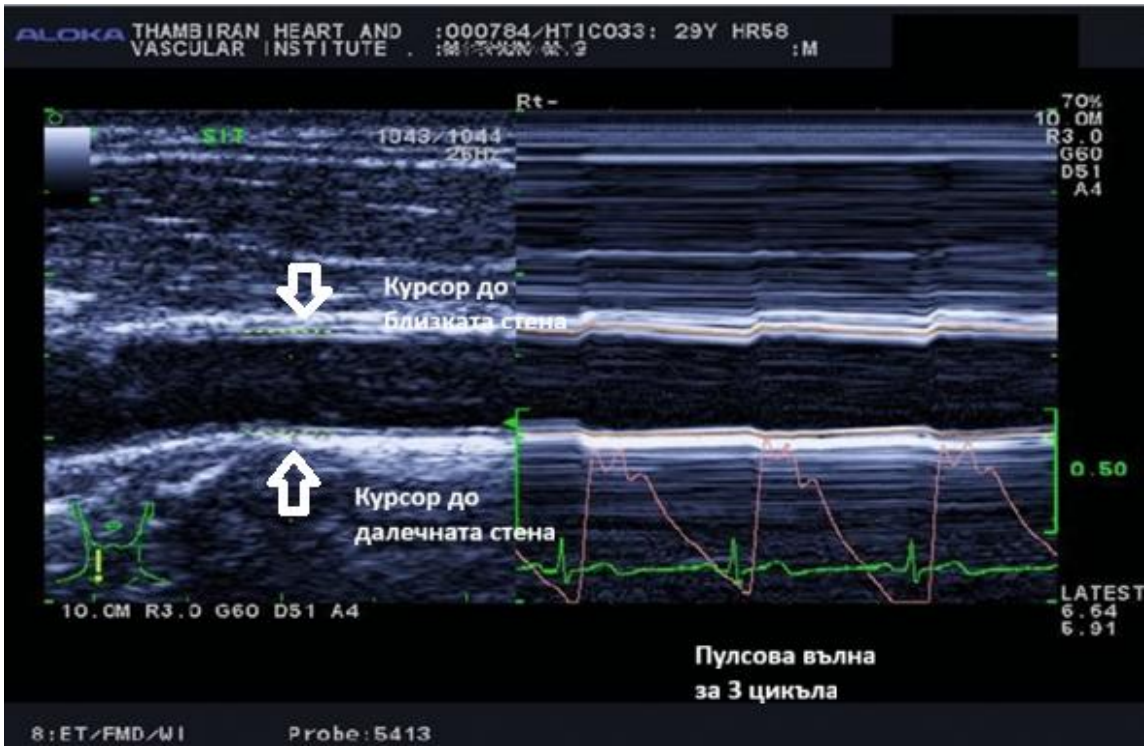


Figure 1

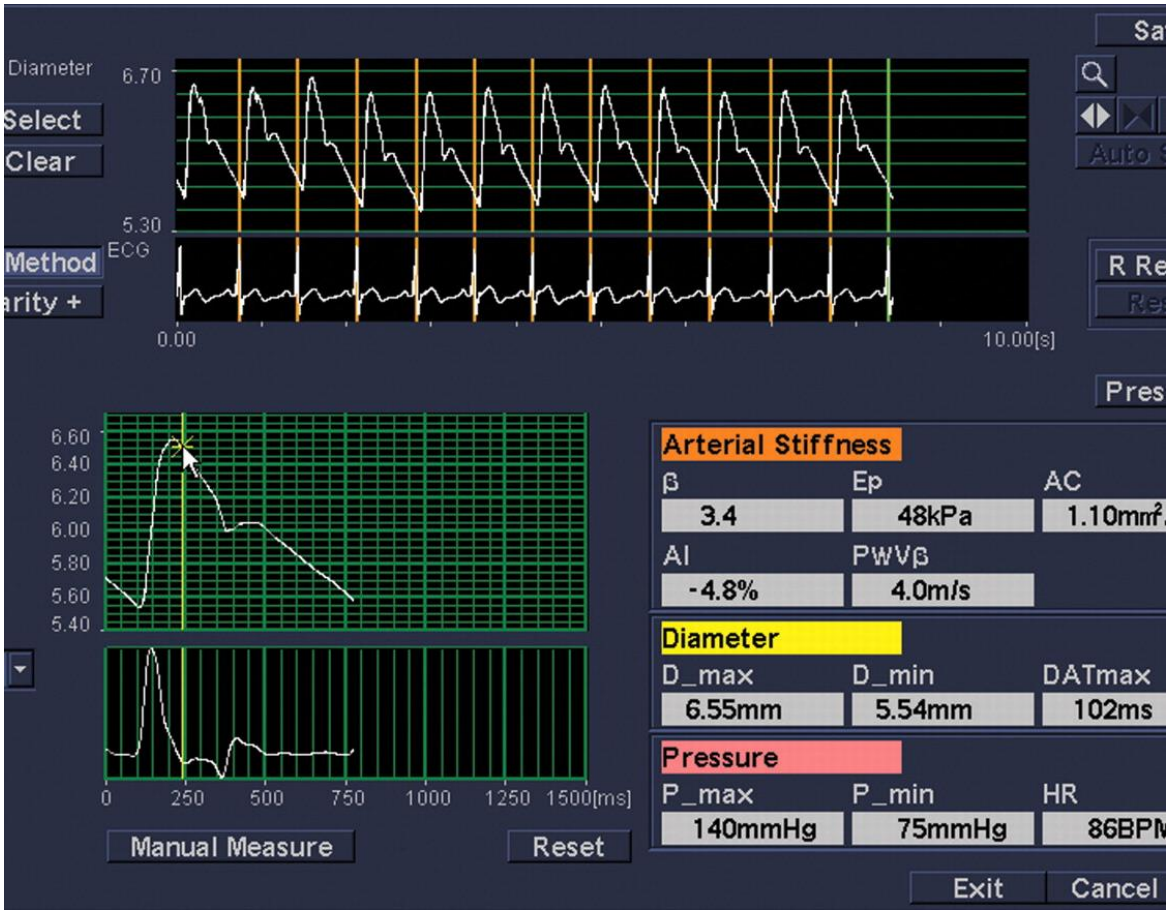


Figure 2

The following hemodynamic and mechanical parameters of the arterial wall were calculated automatically by the device software, based on real-time dynamic changes in arterial diameter and the corresponding blood pressure measurements:

7.1. Pulse Wave Velocity (PWVβ)

$PWV\beta = \sqrt{(\beta \times Pmin / 2\rho)}$

where:

- β – stiffness parameter
- Pmin – diastolic blood pressure
- $\rho = 1050\ kg/m^3$ – blood density

PWVβ reflects the velocity at which the pulse wave propagates along the artery; higher values indicate increased arterial stiffness and elevated cardiovascular risk.

7.2. β-Stiffness Index (β)

$\beta = \ln(P_{max} / P_{min}) / ((D_{max} - D_{min}) / D_{min})$
where:

- P_{max} – systolic blood pressure
- P_{min} – diastolic blood pressure
- D_{max}, D_{min} – maximal and minimal arterial diameter

This index assesses the sensitivity of the arterial wall to pressure changes. Higher values correspond to reduced vascular elasticity.

7.3. Augmentation Index (AIx)

$AIx = (\Delta P / P) \times 100$
where:

- ΔP – difference between the second and first systolic peaks (reflected pressure wave)
- P – pulse pressure (systolic minus diastolic pressure)

AIx quantifies the magnitude of pulse wave reflection and is associated with arterial aging and central hemodynamic load.

7.4. Arterial Compliance (AC)

$AC = \pi(D_{max}^2 - D_{min}^2) / 4(P_{max} - P_{min})$
where:

- P_{max}, P_{min} – maximal and minimal blood pressures
- D_{max}, D_{min} – maximal and minimal arterial diameters
- $4/\pi$ – constant ensuring correct geometric normalization

AC reflects the ability of the artery to expand in response to rising intraluminal pressure; lower values indicate a stiffer, less compliant arterial wall.

7.5. Elastic Modulus (Ep)

$Ep = ((D_{max} - D_{min}) / D_{min}) / (P_{max} - P_{min})$
where:

- D_{max}, D_{min} – maximal and minimal arterial diameter
- P_{max}, P_{min} – systolic and diastolic blood pressure

The numerator, (Dmax − Dmin) / Dmin, represents the relative arterial diameter change during the cardiac cycle, while the denominator, Pmax − Pmin, corresponds to pulse pressure—the driving force for arterial dilation.
Higher Ep values indicate a stiffer vascular wall requiring greater force to produce a given degree of deformation.

These parameters provide a comprehensive, non-invasive assessment of arterial function and are instrumental for detecting early vascular alterations.

8. Statistical Analysis

Statistical analysis of the collected data was performed using the specialized software Jamovi, version 2.6.23 (The jamovi project, 2024; https://www.jamovi.org). The selection of specific statistical methods was determined by the type of variables, the distribution of the data, and the number of groups being compared. The primary objective of the analysis was to identify statistically and clinically significant differences among the studied groups with respect to demographic, clinical, laboratory, and vascular parameters.

8.1. **Descriptive Statistics**

To provide a basic characterization of the studied data, a descriptive statistical analysis was performed, tailored to the type of variables. For continuous quantitative variables, the following parameters were calculated: mean (Mean) as a measure of central tendency, standard deviation (SD) as a measure of dispersion and variability, minimum and maximum values to describe the range of distribution, and 95% confidence interval (CI) to assess the statistical reliability of the mean. Categorical variables were described using absolute frequency (n) and relative frequency (%).

8.2. **Between-Group Comparisons**

Differences among the three main study groups—patients treated with TNF inhibitors, upadacitinib, and healthy controls—with respect to continuous variables (age, BMI, lipid profile values, vascular parameters, etc.) were assessed using one-way analysis of variance (ANOVA). Prior to performing ANOVA, homogeneity of variances was evaluated using Levene's test. When ANOVA yielded a statistically significant result ($p < 0.05$), post hoc multiple comparison tests were applied to identify pairwise group differences. Tukey's HSD test was used when variance homogeneity was confirmed, while the Games–Howell test was applied in cases of violated homogeneity.

For comparisons between two independent groups (e.g., TNF inhibitors vs. upadacitinib), the choice of statistical method depended on the normality of the data distribution. When a normal distribution was confirmed, an independent samples t-test was used. In cases of non-normal distribution or unequal variances, the Mann–Whitney U test was applied as a nonparametric alternative. Normality of distribution was assessed using the Shapiro–Wilk test, as well as by visual inspection (Q–Q plots and histograms).

For the analysis of categorical variables (sex, smoking status, prior biologic therapy, concomitant therapy), the chi-square test (χ^2) was used. When expected cell frequencies were small ($n < 5$), Fisher's exact test was applied.

To enable a more refined interpretation of statistically significant differences, effect size measures were calculated. For independent samples t-tests, Cohen's d was used, with interpretation as follows:

- $d \approx 0.2$ — small effect
- $d \approx 0.5$ — moderate effect
- $d \geq 0.8$ — large effect

For the Mann–Whitney U test, the rank-biserial correlation (r) was used:

- $r \approx 0.1$ — weak effect
- $r \approx 0.3$ — moderate effect
- $r \geq 0.5$ — strong effect

In clinical practice, small effect sizes are generally considered to reflect limited clinical significance, whereas moderate and large effect sizes suggest more meaningful and clinically relevant differences that may influence clinical decision-making or therapeutic choice. The calculation of effect sizes allows for a more informed interpretation of the clinical relevance of the results, even in the absence of statistical significance.

8.3. Correlation Analysis

To evaluate the relationships between quantitative variables in the study, two types of correlation analyses were applied depending on data characteristics. For variables with approximately normal distributions, Pearson's correlation coefficient (Pearson's r) was used to assess the strength of linear associations between two continuous variables. The values of the

correlation coefficient range from –1 to +1, with values close to ±1 indicating strong negative or positive linear relationships, and values close to zero indicating the absence of a linear association. When variables did not follow a normal distribution or were expressed on a rank scale, Spearman's rank correlation coefficient (Spearman's rho) was applied. This nonparametric method assesses the degree of monotonic association between two variables without assuming a linear relationship. As with Pearson's r, values range from –1 to +1, with positive values indicating direct and negative values indicating inverse relationships. This approach provides greater flexibility for the analysis of real-world clinical data, which often do not meet normality assumptions.

8.4. Regression Analysis

To evaluate the relationship between type of therapy and parameters of arterial stiffness as well as the endothelial dysfunction marker ADMA, a multivariable linear regression analysis was performed. The analyses aimed to determine whether treatment with a JAK inhibitor (upadacitinib) or a TNF inhibitor exerted a significant effect on arterial stiffness and endothelial dysfunction parameters compared with healthy controls, and whether these parameters were influenced by disease-related variables. Treatment group was included as a categorical independent variable with three levels (upadacitinib, TNF inhibitor, healthy controls). In the first regression model, the control group served as the reference category, while in the second model the TNF inhibitor group was used as the reference category. Separate regression models were constructed for each dependent variable: pulse wave velocity (PWV), β-stiffness index, augmentation index (AIx, %), arterial compliance (AC), elastic modulus (Ep), and ADMA. Two regression models were developed. The first model included general covariates common to all three groups: age, sex, body mass index, smoking status, and lipid profile parameters, and was used to compare both treatment groups with the control group. The second model incorporated disease-related variables: disease duration, duration of therapy, previous biologic treatment, corticosteroid use, ESR, CRP, and disease activity indices, and was used to compare the two treatment groups. Regression coefficients (β), p-values, 95% confidence intervals, and the coefficient of determination (R^2) were calculated. Model assumptions, including linearity, homoscedasticity, and normality of residuals, were assessed.

8.5. Criteria for Statistical Significance

In all analyses, the threshold for statistical significance was set at $p < 0.05$, using two-tailed testing. P-values are reported to three decimal places. Values of $p < 0.001$ are reported as $p < 0.001$.

IV. Results

1. General Characteristics of the Treatment Groups and Controls

1.1 Distribution of Patients Across the Three Study Groups

Table. 2

Groupe	N	%
TNF	41	37.6%
Upadacitinib	38	34.9%
Control	30	27.5%

Note: TNF = tumor necrosis factor inhibitor group; *n* = number of participants; % = percentage of the total study population.

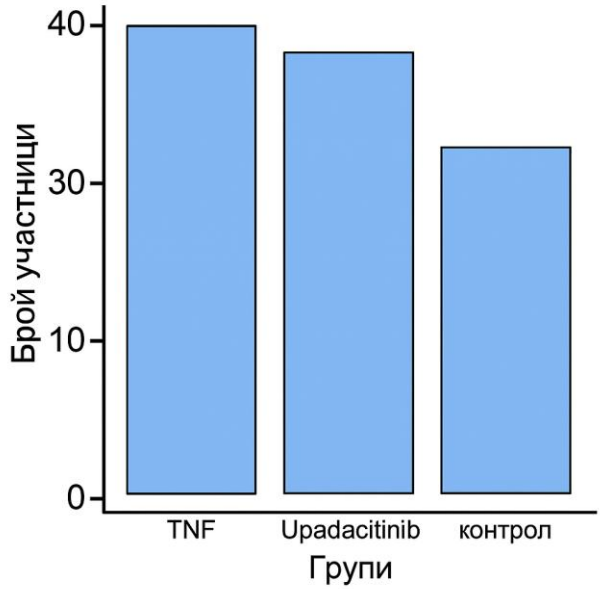


Fig. 3. Distribution of Patients Across the Three Study Groups

1.2. Sex Distribution

Table. 3

Group	Sex	Female (n, %)	Male (n, %)	Total (n, %)
TNF		33 (80.5%)	8 (19.5%)	41 (100.0%)
Upadacitinib		34 (89.5%)	4 (10.5%)	38 (100.0%)
Control		23 (76.7%)	7 (23.3%)	30 (100.0%)
Total		90 (82.6%)	19 (17.4%)	109 (100.0%)

Note: *$\chi^2 = 2.11$, df = 2, p = 0.348. TNF = tumor necrosis factor inhibitor group.*

In the TNF inhibitor group, a total of 41 participants were included, of whom 80.5% were women (n = 33) and 19.5% were men (n = 8). In the upadacitinib-treated group, women accounted for 89.5% (n = 34), while men represented 10.5% (n = 4). In the control group, women comprised 76.7% (n = 23) and men 23.3% (n = 7). The results of the chi-square test did not demonstrate statistically significant differences between the groups with regard to sex distribution ($\chi^2 = 2.11$, df = 2, p = 0.348).

1.3. Age

The mean age of participants across the three groups—those treated with TNF inhibitors (n = 41), upadacitinib (n = 38), and the control group (n = 30)—did not differ significantly (F = 0.568, df = 2, p = 0.568). The mean ages were 56.4 years (SD = 13.5), 55.6 years (SD = 10.1), and 53.5 years (SD = 10.2), respectively, indicating homogeneity among the groups with respect to this demographic parameter.

Table 4. Mean age of participants by study group. Note: TNF = tumor necrosis factor inhibitor group.

Variable	Group	N	Mean age (years)	Standard deviation	ANOVA (F / p)
Age	TNF inhibitors	41	56.4	13.5	F = 0.568, df = 2, p = 0.568
	Upadacitinib	38	55.6	10.1	
	Control	30	53.5	10.2	

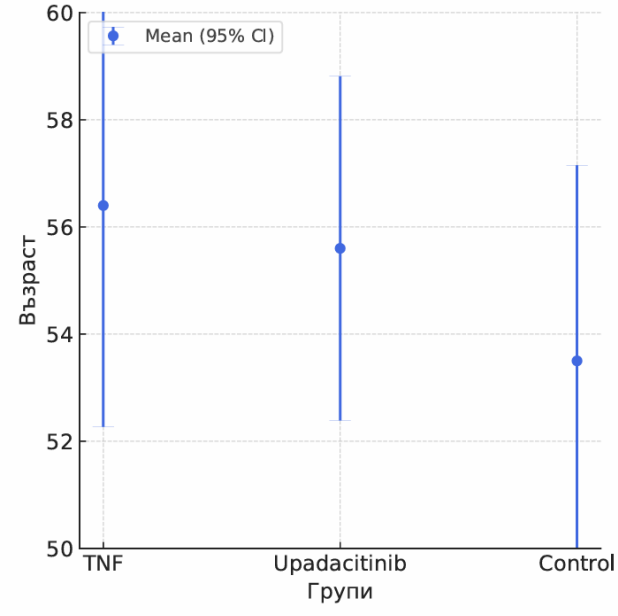


Fig. 4. Mean age of participants by group. Note: TNF = tumor necrosis factor inhibitor group; CI = confidence interval.

1.4. Body Mass Index (BMI)

Analysis of BMI using one-way ANOVA revealed no statistically significant differences among patients treated with TNF inhibitors (n = 41), those treated with upadacitinib (n = 38), and the control group (n = 30) (F = 0.103, df = 2, p = 0.903). Post hoc Tukey analysis confirmed the absence of significant differences between any of the groups: TNF inhibitors vs. upadacitinib (mean difference = −0.179, p = 0.985), TNF inhibitors vs. controls (mean difference = −0.522, p = 0.894), and upadacitinib vs. controls (mean difference = −0.342, p = 0.954).

Table 5. Mean body mass index (BMI) and standard deviation by study groups. Note: TNF = tumor necrosis factor inhibitor group; BMI = body mass index.

Parameter	*Group*	*N*	*Mean value*	*Standard deviation*	*ANOVA (F/p)*
BMI	*TNF*	*41*	*25.5*	*4.25*	*F = 0.103, df = 2, p = 0.903*
	Upadacitinib	*38*	*25.7*	*4.92*	
	Control	*30*	*26.0*	*5.39*	

Table 6. Tukey post-hoc test for between-group comparisons of body mass index (BMI). Note: *p < 0.05, p < 0.01, p < 0.001. BMI = body mass index.*

Comparison	**TNF**	**Upadacitinib**	**Control**
TNF – Mean difference	—	−0.179	−0.522
p-value	—	0.985	0.894
Upadacitinib – Mean difference		—	−0.342
p-value		—	0.954
Control – Mean difference			—
p-value			—

1.5. Smoking Status

The proportion of current smokers was highest in the Upadacitinib group at 50.0% (n=19), followed by the TNF inhibitor group at 43.9% (n=18), and was lowest in the control group at 33.3% (n=10). Non-smokers constituted 56.1% in the TNF group, 50.0% in the Upadacitinib

group, and 66.7% among controls. The differences between the groups did not reach statistical significance (χ^2=1.92, df=2, p=0.384).

Table 7. Distribution of participants according to current smoking status. Note: χ^2 = 1.92, df = 2, p = 0.384. TNF = tumor necrosis factor inhibitor group.

Group	N	No (n, %)	Yes (n, %)	Total (n, %)
TNF	41	23 (56.1%)	18 (43.9%)	41 (100.0%)
Upadacitinib	38	19 (50.0%)	19 (50.0%)	38 (100.0%)
Control	30	20 (66.7%)	10 (33.3%)	30 (100.0%)
Total	109	62 (56.9%)	47 (43.1%)	109 (100.0%)

1.6. Lipid Profiles

A comparative analysis of the lipid profile among the three study groups—patients treated with TNF inhibitors (n = 41), patients treated with upadacitinib (n = 38), and healthy controls (n = 30)—revealed statistically significant differences in total cholesterol, LDL cholesterol, and HDL cholesterol levels, but not in triglyceride levels.

For total cholesterol, a significant effect of group membership was observed (F = 7.576, df = 2, p = 0.001). Tukey post hoc testing showed that the upadacitinib group had significantly higher total cholesterol values compared with both the TNF inhibitor group (mean difference = 0.927, p = 0.002) and the control group (mean difference = 0.954, p = 0.004), whereas no significant difference was found between the TNF inhibitor group and the controls (p = 0.995).

Similarly, LDL cholesterol levels differed significantly among the groups (F = 4.488, df = 2, p = 0.013). Patients treated with upadacitinib exhibited significantly higher LDL cholesterol compared with those receiving TNF inhibitors (mean difference = 0.615, p = 0.028) and the control group (mean difference = 0.651, p = 0.033). No significant difference was observed between the TNF inhibitor group and the controls (p = 0.989).

HDL cholesterol levels also showed statistically significant between-group differences (F = 3.979, df = 2, p = 0.022). Tukey post hoc analysis demonstrated higher HDL cholesterol values in the upadacitinib group compared with both the TNF inhibitor group (mean difference = 0.256, p = 0.049) and the control group (mean difference = 0.287, p = 0.040), with no significant difference between the TNF inhibitor group and the controls (p = 0.961).

In contrast, no statistically significant differences were observed in triglyceride levels among the three groups (F = 0.770, df = 2, p = 0.402). The Games–Howell post hoc test confirmed the absence of significant differences across all pairwise comparisons (all p > 0.4).

Table 8. Lipid profile parameters across the study groups. Note: TNF = tumor necrosis factor inhibitor group; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

Parameter (mmol/L)	Group	N	Mean	Standard Deviation	ANOVA (F / p)
Total cholesterol	TNF	41	5.35	1.093	F = 7.576, df = 2, p = 0.001
	Upadacitinib	38	6.28	1.207	
	Control	30	5.33	1.325	
LDL cholesterol	TNF	41	3.13	0.965	F = 4.488, df = 2, p = 0.013
	Upadacitinib	38	3.74	1.087	
	Control	30	3.09	1.106	
HDL cholesterol	TNF	41	1.57	0.513	F = 3.979, df = 2, p = 0.022
	Upadacitinib	38	1.83	0.427	
	Control	30	1.54	0.487	
Triglycerides	TNF	41	1.31	0.538	F = 0.770, df = 2, p = 0.402
	Upadacitinib	38	1.51	0.866	
	Control	30	1.46	0.856	

Table 9. Tukey post-hoc test for pairwise comparisons of total cholesterol between study groups. *Note: TNF = tumor necrosis factor inhibitor group. *p < 0.01.

Comparison	TNF	Upadacitinib	Control
TNF – Mean difference	—	−0.927**	0.027
p-value	—	0.002	0.995
Upadacitinib – Mean difference		—	0.954**
p-value		—	0.004
Control – Mean difference			—
p-value			—

Table 10. Tukey post-hoc test for pairwise comparisons of LDL cholesterol between study groups. *Note: TNF = tumor necrosis factor inhibitor group. p < 0.05.

Comparison	TNF	Upadacitinib	Control
TNF – Mean difference	—	−0.615*	0.036
p-value	—	0.028	0.989
Upadacitinib – Mean difference		—	0.651*
p-value		—	0.033

Control – Mean difference			—
p-value			—

Table 11. Tukey post-hoc test comparing HDL levels between groups. Note: p < .05, p < .01, p < .001. TNF = tumor necrosis factor inhibitor group.

Comparison	TNF	Upadacitinib	Control
TNF – Mean difference	—	−0.256*	0.031
p-value	—	0.049	0.961
Upadacitinib – Mean difference		—	0.287*
p-value		—	0.040
Control – Mean difference			—
p-value			—

Table 12. Games–Howell post-hoc test comparing triglyceride levels between groups. Note: p < .05, p < .01, p < .001. TNF = tumor necrosis factor inhibitor group.

Comparison	TNF	Upadacitinib	Control
TNF – Mean difference	—	−0.204	−0.152
p-value	—	0.432	0.672
Upadacitinib – Mean difference		—	0.052
p-value		—	0.967
Control – Mean difference			—
p-value			—

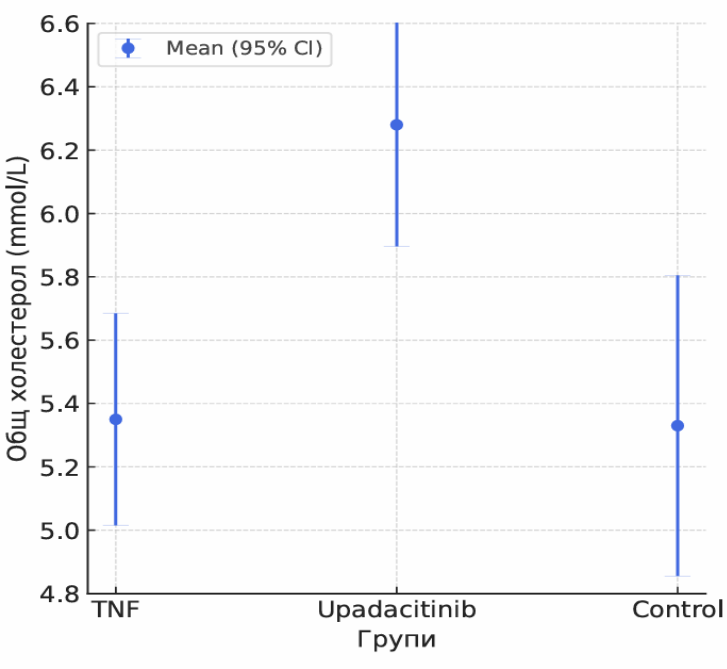


Figure 5. Comparison of the groups by total cholesterol.Note: TNF = tumor necrosis factor inhibitor group. CI = confidence interval.

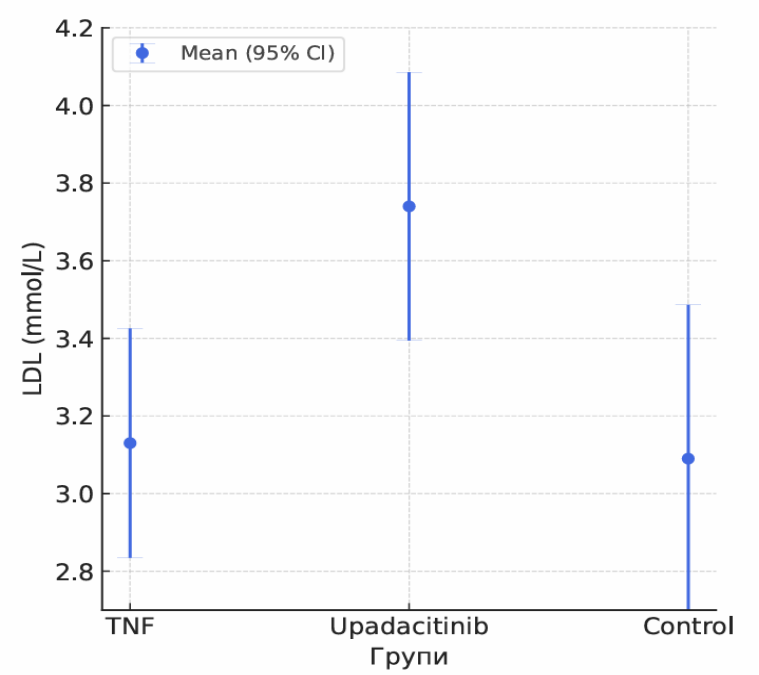


Figure 6. Comparison of the groups by LDL cholesterol. Note: TNF = tumor necrosis factor inhibitor group. CI = confidence interval.

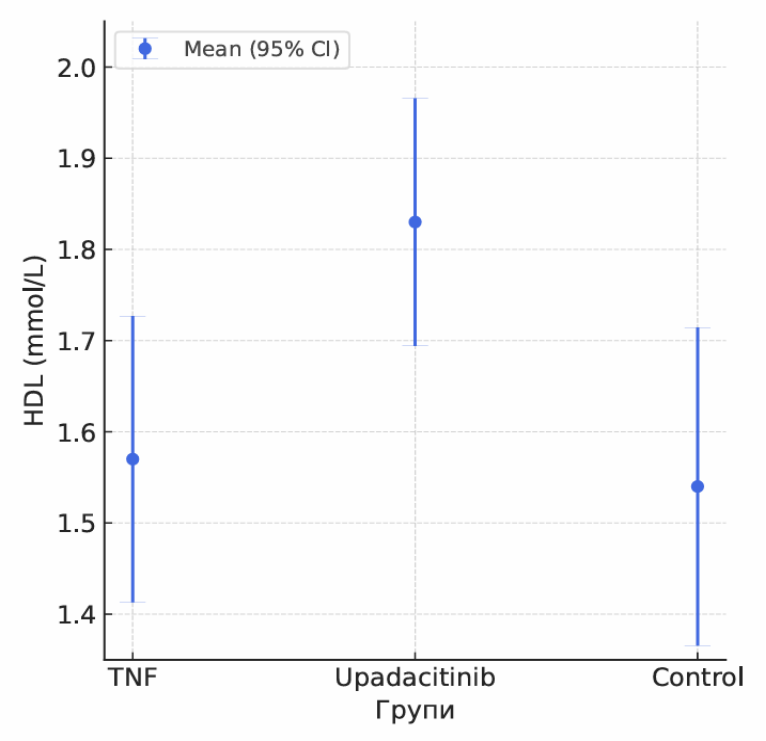


Figure 7. Comparison of the groups by HDL cholesterol. Note: TNF = tumor necrosis factor inhibitor group. CI = confidence interval.

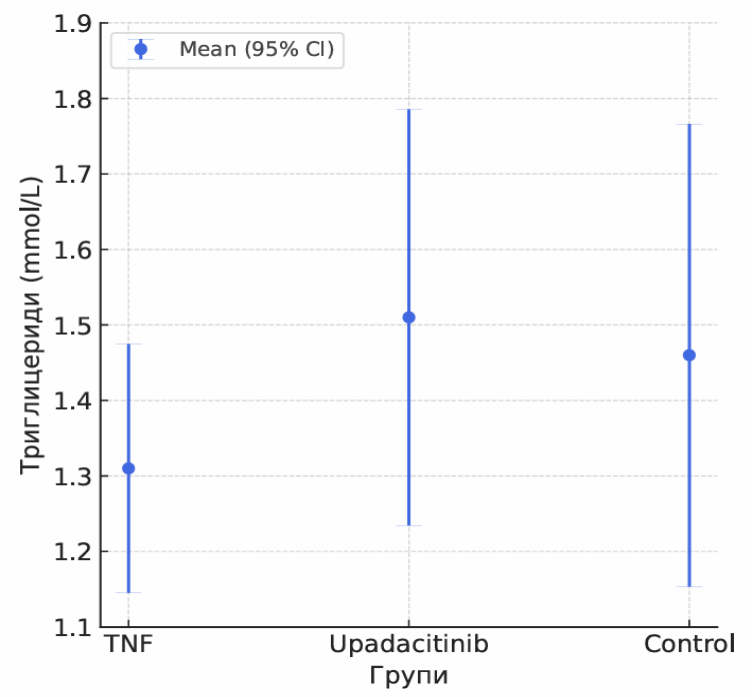


Figure 8. Comparison of the groups by triglyceride levels. Note: TNF = tumor necrosis factor inhibitor group. CI = confidence interval.

1.7. Assessment of Cardiovascular Risk Using the Framingham Risk Score (FRS)

The mean 10-year Framingham risk for myocardial infarction or cardiovascular death was comparable across the three groups: 4.39% (SD = 4.99) in patients treated with TNF inhibitors, 4.26% (SD = 4.10) in the upadacitinib group, and 3.93% (SD = 4.09) in the control group. One-way analysis of variance (ANOVA) demonstrated no statistically significant differences between the groups (F = 0.09, df = 2, p = 0.91).

Table 13. Mean 10-year risk of myocardial infarction or death according to the Framingham Risk Score by study group. Note: TNF = tumor necrosis factor inhibitor group; MI = myocardial infarction.

Parameter	Group	N	Mean	Standard Deviation	ANOVA (F/p)
Framingham score – 10-year risk of MI or death (%)	TNF	41	4.39	4.99	F = 0.09, df = 2, p = 0.912
	Upadacitinib	38	4.26	4.10	
	Control	30	3.93	4.09	

The results of the Tukey post hoc test demonstrated no statistically significant differences in the 10-year Framingham risk of myocardial infarction or death between any of the study groups. The mean differences were as follows: between Group 1 (TNF) and Group 2 (upadacitinib), the mean difference was 0.122 (p = 0.992); between Group 1 and Group 3 (control), the mean difference was 0.452 (p = 0.906); and between Group 2 and Group 3, the mean difference was 0.330 (p = 0.951).

Table 14. Tukey post hoc test for comparison between groups according to the Framingham score (10-year risk of myocardial infarction or death). Note: p < 0.05, p < 0.01, p < 0.001. TNF = tumor necrosis factor inhibitor group; MI = myocardial infarction.

Comparison	TNF	Upadacitinib	Control
TNF – Mean difference	—	0.122	0.452
p-value	—	0.992	0.906
Upadacitinib – Mean difference		—	0.330
p-value		—	0.951
Control – Mean difference			—
p-value			—

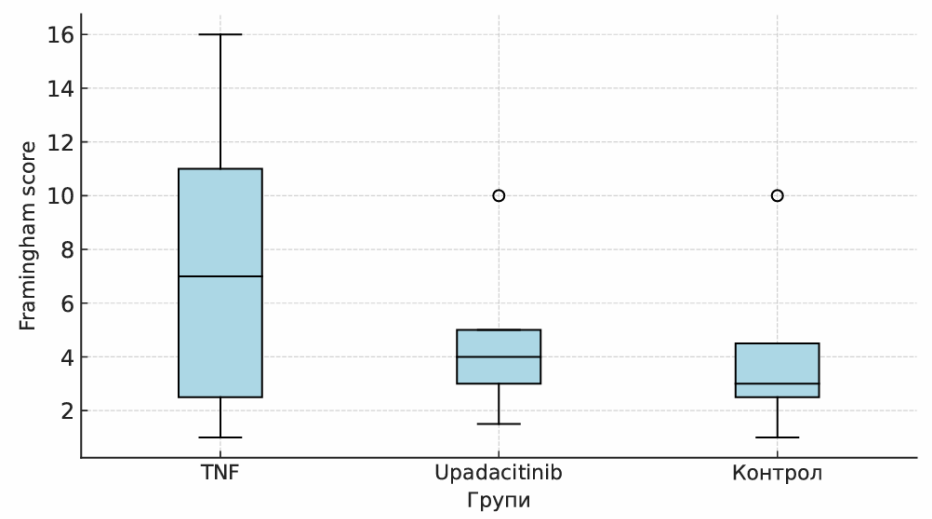


Figure 9. Comparison between groups according to the Framingham score (10-year risk of myocardial infarction or death).

2. Disease-Related Parameters

2.1. Disease Duration, Duration of Therapy, and Previous Biological Treatments

A comparative analysis between the groups treated with TNF inhibitors and upadacitinib, using the Mann–Whitney U test, revealed no statistically significant difference in disease duration (TNF: M = 11.81 years, SD = 7.15; upadacitinib: M = 11.53 years, SD = 7.44; U = 760, p = 0.856), with a small effect size (r = −0.0244). In contrast, the duration of the current treatment was significantly shorter in the upadacitinib group compared with the TNF inhibitor group (TNF: M = 5.34 years, SD = 4.00; upadacitinib: M = 2.07 years, SD = 2.00; U = 442, $p < 0.001$), with a moderate effect size (r = 0.4332). This difference is attributable to the fact that TNF inhibitors represent an older and well-established therapeutic class that has been used for a longer period in Bulgarian rheumatology practice, whereas upadacitinib has been in clinical use only since 2019. Furthermore, the number of previously administered biological agents was significantly higher in the upadacitinib group (M = 0.74, SD = 0.95) compared with the TNF inhibitor group (M = 0.37, SD = 0.73; U = 575, p = 0.020; χ^2 = 10.2, df = 4, p = 0.037), with a moderate effect size (r = 0.2625). This finding suggests that patients receiving upadacitinib had undergone more extensive prior biologic treatments, possibly reflecting a more aggressive disease course that necessitated multiple therapeutic switches.

Table 15. Comparison of disease duration, treatment duration and previous biological therapies between the groups (Mann–Whitney U test)

Variable	Group	N	Mean	SD	Mann–Whitney U / p	Effect size (rank biserial correlation)
Disease duration (years)	TNF	41	11.81	7.15	U = 760, p = 0.856	-0.024
	Upadacitinib	38	11.53	7.44		
Treatment duration (years)	TNF	41	5.34	4.00	U = 442, p < 0.001	0.433
	Upadacitinib	38	2.07	2.00		
Number of previous biological therapies	TNF	41	0.37	0.73	U = 575, p = 0.020	0.263
	Upadacitinib	38	0.74	0.95		

Table 16. Distribution of patients according to the number of previous biological therapies (χ^2 test)
Note: $\chi^2 = 10.2$, df = 4, p = 0.037.

Group	0 n (%)	1 n (%)	2 n (%)	3 n (%)	4 n (%)	Total n (%)
TNF	31 (75.6%)	6 (14.6%)	3 (7.3%)	1 (2.4%)	0 (0.0%)	41 (100%)
Upadacitinib	18 (47.4%)	16 (42.1%)	1 (2.6%)	2 (5.3%)	1 (2.6%)	38 (100%)
Total	49 (62.0%)	22 (27.8%)	4 (5.1%)	3 (3.8%)	1 (1.3%)	79 (100%)

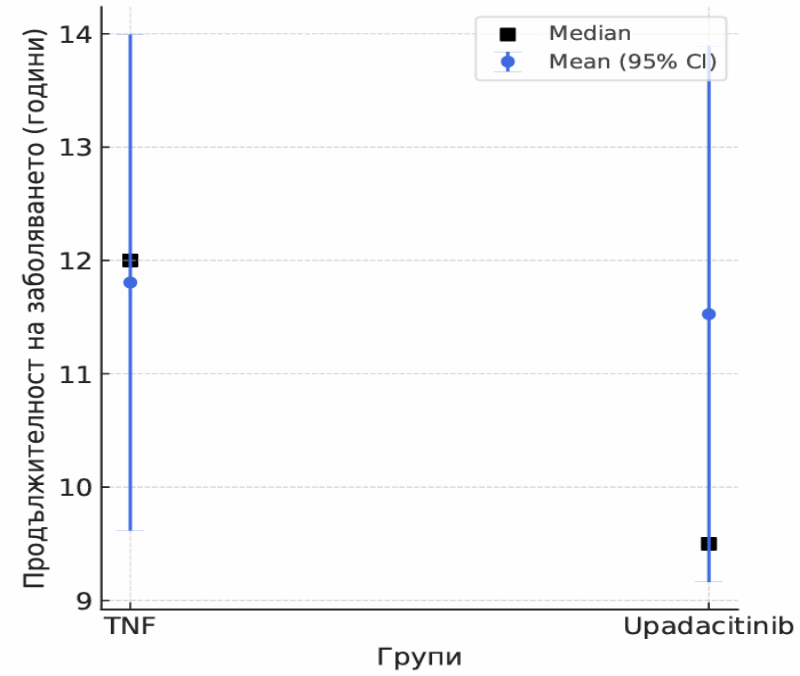


Figure 10. Comparison of disease duration between the study groups.

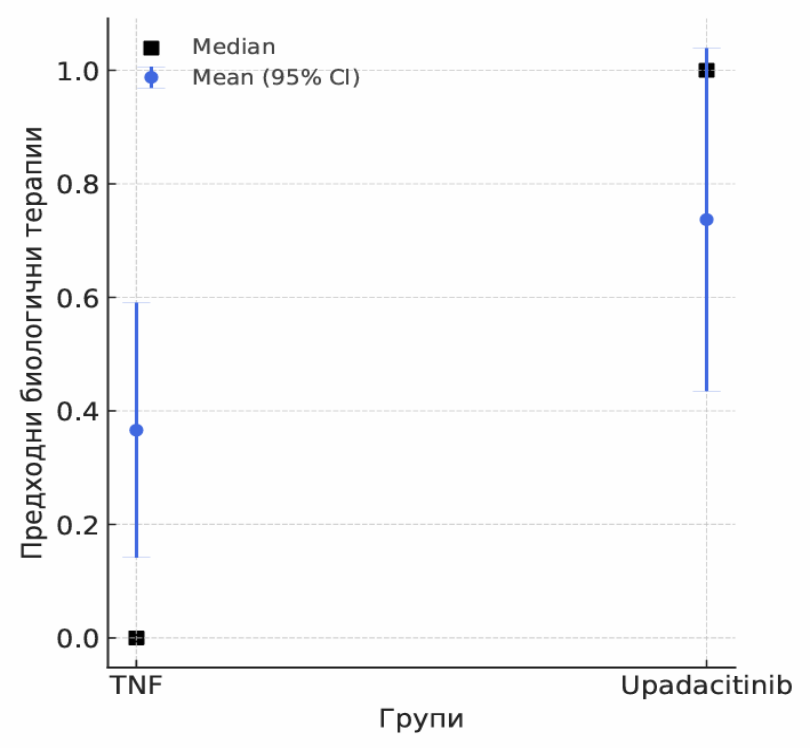


Figure 11. Comparison of the number of previously used biologic therapies between the study groups.

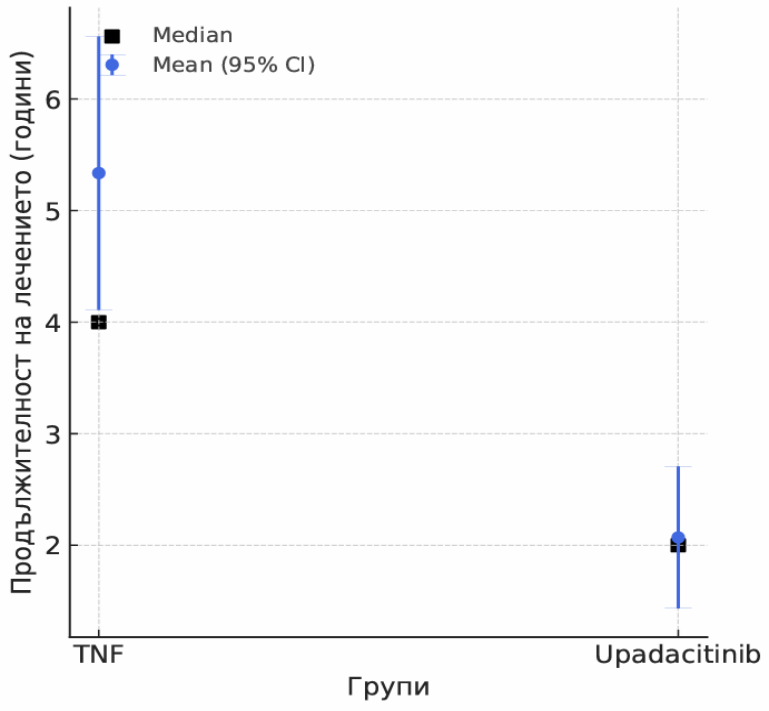


Figure 12. Duration of treatment.

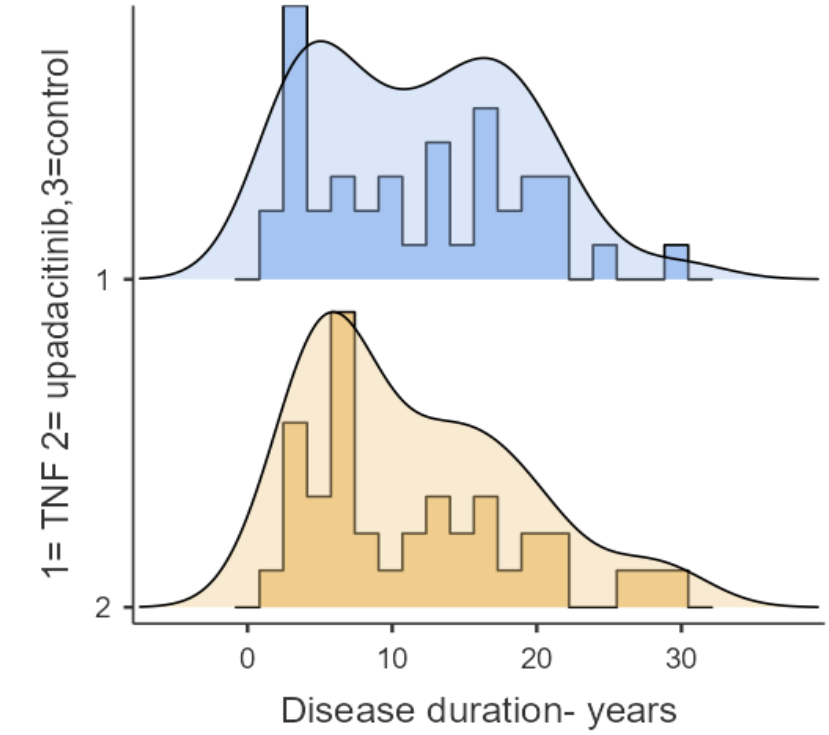


Figure 13. Histogram of disease duration in years.

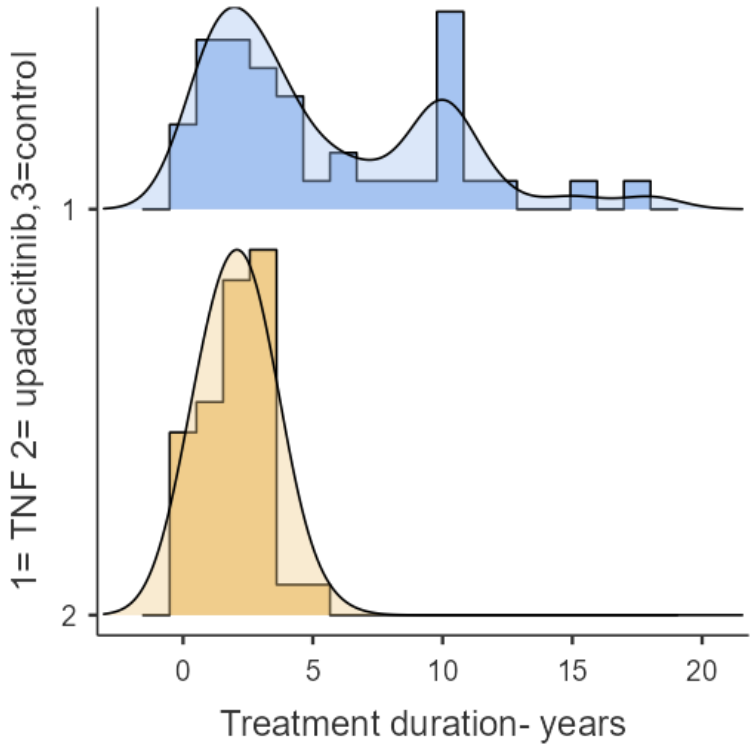


Figure 14. Histogram of treatment duration (in years).

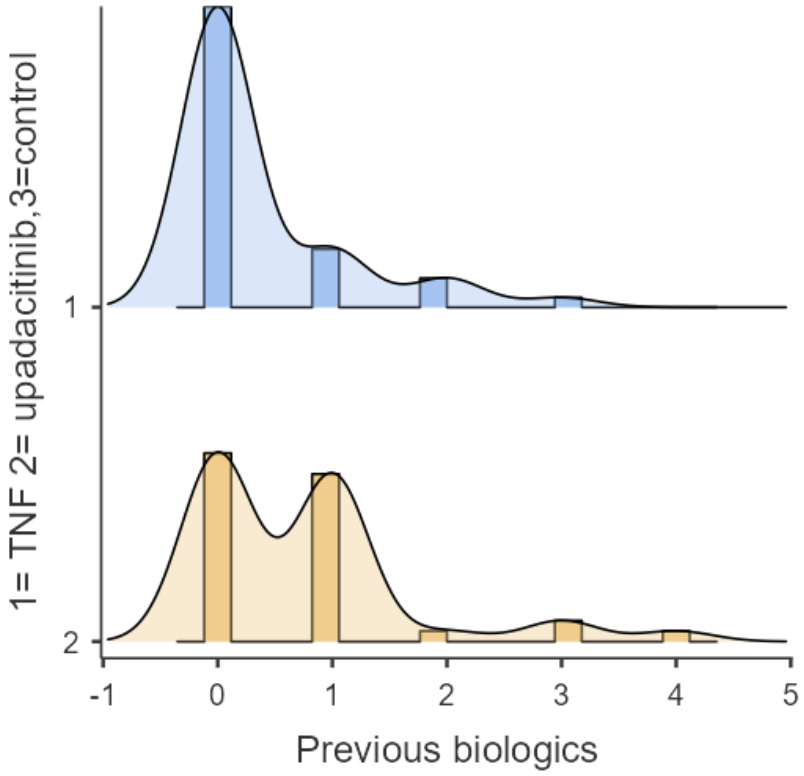


Figure 15. Histogram of prior biological therapies.

2.2 Use of corticosteroids and conventional synthetic disease-modifying antirheumatic drugs

The two treatment groups did not differ significantly with respect to the dose of administered corticosteroids, expressed as prednisolone equivalent (TNF: M = 1.31 mg, SD = 2.96; upadacitinib: M = 1.65 mg, SD = 2.68; U = 709, p = 0.379), with a small effect size (r = 0.0899).

In contrast, the distribution of concomitant conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) differed significantly between the two groups ($\chi^2 = 19.2$, df = 4, $p < 0.001$). In the TNF inhibitor group, the most commonly used concomitant medication was methotrexate (MTX) in 53.7% (n = 22) of patients, followed by patients receiving no concomitant therapy (41.5%, n = 17). A small proportion of patients were treated with azathioprine (AZA) – 4.9% (n = 2).

In the upadacitinib group, the majority of patients did not receive concomitant csDMARD therapy (78.9%, n = 30), while the remaining patients were treated with MTX (13.2%, n = 5), hydroxychloroquine (HCQ) (5.3%, n = 2), and AZA (2.6%, n = 1).

These findings highlight differences in therapeutic strategies between the groups, with more frequent use of monotherapy in the upadacitinib group. This approach is most likely driven by the established efficacy of upadacitinib as monotherapy and by EULAR recommendations for the use of JAK inhibitors in patients with intolerance to methotrexate.

Table 17. Mean corticosteroid dose (prednisolone equivalent) – comparison between groups (Mann–Whitney U test) Note: TNF = tumor necrosis factor inhibitor group.

Variable	Group	N	Mean	SD	Mann–Whitney U / p	Effect Size (Rank biserial correlation)
Steroid dose (prednisolone equivalent, mg)	TNF	41	1.31	2.96	U = 709, p = 0.379	0.090
	Upadacitinib	38	1.65	2.68		

Table 18. Distribution of patients according to concomitant DMARD therapy (χ^2 test) Note: DMARD = Disease-Modifying Antirheumatic Drug; A = Leflunomide; AZA = Azathioprine; HCQ = Hydroxychloroquine; MTX = Methotrexate; N = None; $\chi^2 = 19.2$, df = 4, $p < 0.001$.

Group	A (n, %)	AZA (n, %)	HCQ (n, %)	MTX (n, %)	N (n, %)	Total (n, %)
TNF	2 (4.9%)	0 (0.0%)	0 (0.0%)	22 (53.7%)	17 (41.5%)	41 (100.0%)
Upadacitinib	0 (0.0%)	1 (2.6%)	2 (5.3%)	5 (13.2%)	30 (78.9%)	38 (100.0%)
Total	2 (2.5%)	1 (1.3%)	2 (2.5%)	27 (34.2%)	47 (59.5%)	79 (100.0%)

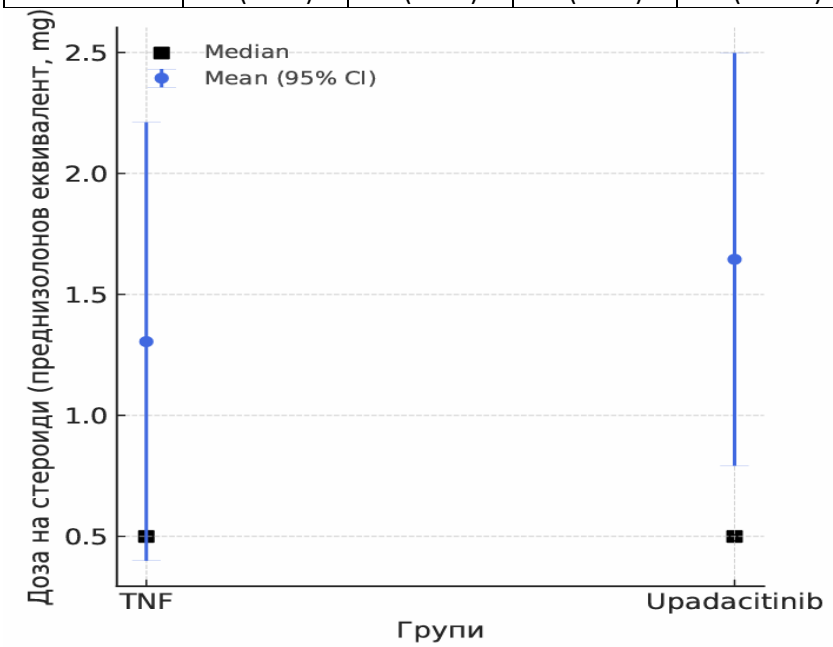


Figure 16. Glucocorticoid dose expressed as prednisolone equivalent

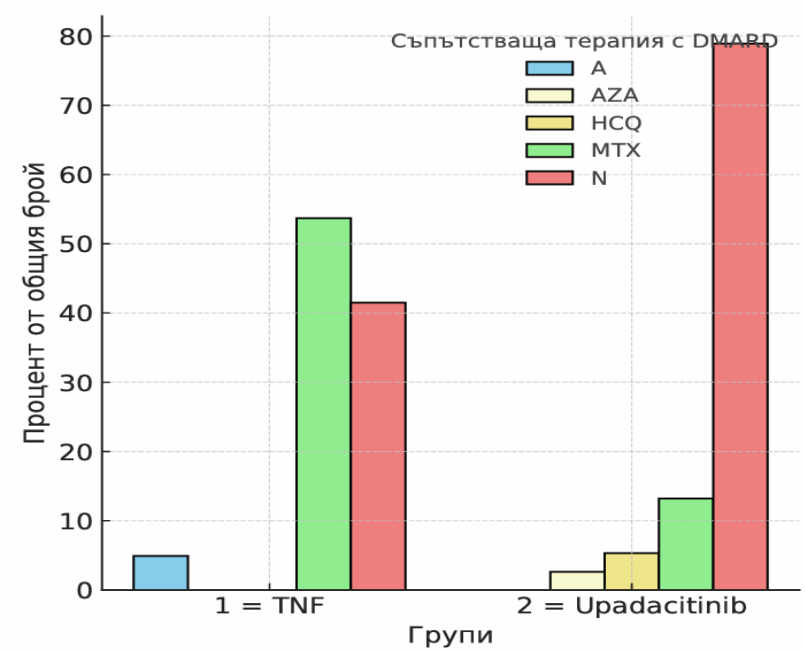


Figure 17. Distribution of patients according to concomitant DMARD therapy.
Note: DMARD = Disease-Modifying Antirheumatic Drugs; A = Leflunomide; AZA = Azathioprine; HCQ = Hydroxychloroquine; MTX = Methotrexate; N = None.

2.3. ESR and CRP Levels in the Study Groups

The comparison between the groups treated with TNF inhibitors and upadacitinib with respect to laboratory markers of inflammation did not reveal statistically significant differences. Regarding C-reactive protein (CRP) levels, the TNF inhibitor group had a mean value of 4.51 mg/L (SD = 5.42), while the upadacitinib group exhibited a higher mean value of 8.47 mg/L (SD = 18.1); however, this difference did not reach statistical significance (U = 618, p = 0.114). The effect size was small (r = −0.207). Similarly, erythrocyte sedimentation rate (ESR) values were comparable between the two groups (TNF inhibitors: M = 34.95 mm/h, SD = 21.16; upadacitinib: M = 33.54 mm/h, SD = 28.1), with no statistically significant difference observed (U = 692, p = 0.393) and a weak effect size (r = −0.112).

Table 19. Comparison of Inflammatory Markers (CRP and ESR) Between the Groups (Mann–Whitney U Test)

Parameter	Group	N	Mean	SD	Mann–Whitney U / p	Effect Size (Rank-biserial r)
CRP (mg/L)	TNF inhibitors	41	4.51	5.42	U = 618, p = 0.114	−0.207
	Upadacitinib	38	8.47	18.1		
ESR (mm/h)	TNF inhibitors	41	34.95	21.16	U = 692, p = 0.393	−0.112

	Upadacitinib	38	33.54	28.1		

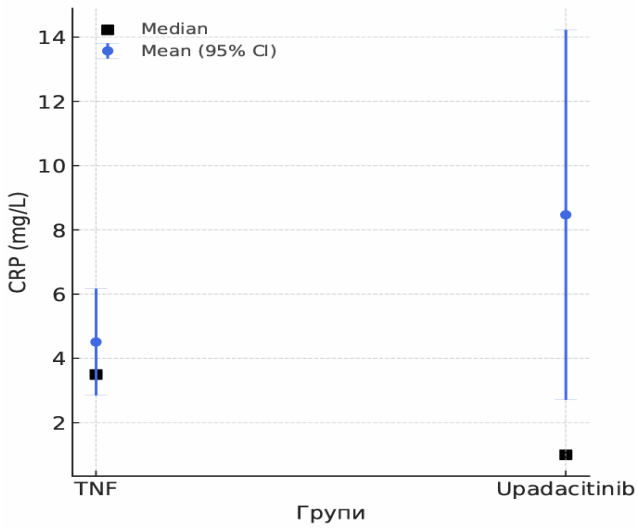


Figure 18. Comparison of CRP Levels Between the Study Groups

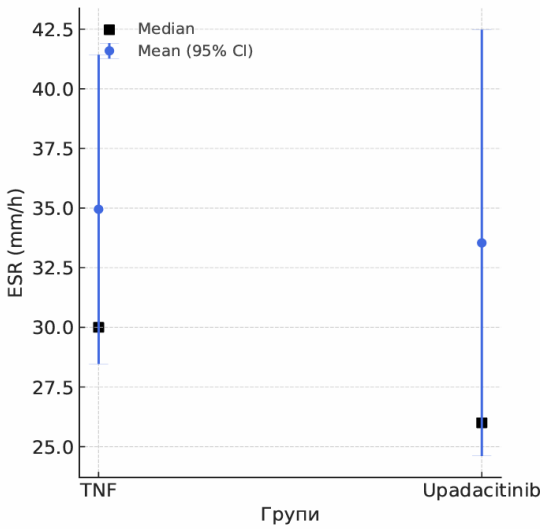


Figure 19. Comparison of ESR Levels Between the Study Groups

2.4. Composite Indices of Disease Activity

The comparative analysis between patients treated with TNF inhibitors and those receiving upadacitinib did not reveal statistically significant differences in disease activity measures. The mean values of the DAS28-ESR index were virtually identical between the two groups (TNF: M = 3.55, SD = 0.91; upadacitinib: M = 3.56, SD = 0.95), with the t-test confirming the absence of a significant difference (t = –0.0865, p = 0.931) and demonstrating a negligible effect size (Cohen's d = –0.0195). Similarly, DAS28-CRP scores did not differ significantly between the two treatment groups (TNF: M = 2.70, SD = 0.78; upadacitinib: M = 2.59, SD = 0.83; U = 686, p = 0.361), with a weak effect size (r = –0.1200). With regard to the Clinical Disease Activity Index (CDAI), although the mean value was slightly higher in the upadacitinib group (M = 10.47, SD = 6.06) compared with the TNF inhibitor group (M = 8.91, SD = 5.59), this difference did not reach statistical significance (U = 678, p = 0.323), and the effect size was again weak (r = 0.1297).

Table 20. Comparison of disease activity indices between the groups (t-test and Mann–Whitney U test)

Index	Group	N	Mean ± SD	Statistical Test / p-value	Effect Size
DAS28-ESR	TNF inhibitors	41	3.55 ± 0.91	*t* = −0.0865, p = 0.931	d = −0.020

Index	Group	N	Mean ± SD	Statistical Test / p-value	Effect Size
	Upadacitinib	38	3.56 ± 0.95		
DAS28-CRP	TNF inhibitors	41	2.70 ± 0.78	U = 686, p = 0.361	r = −0.120
	Upadacitinib	38	2.59 ± 0.83		
CDAI	TNF inhibitors	41	8.91 ± 5.59	U = 678, p = 0.323	r = 0.130
	Upadacitinib	38	10.47 ± 6.06		

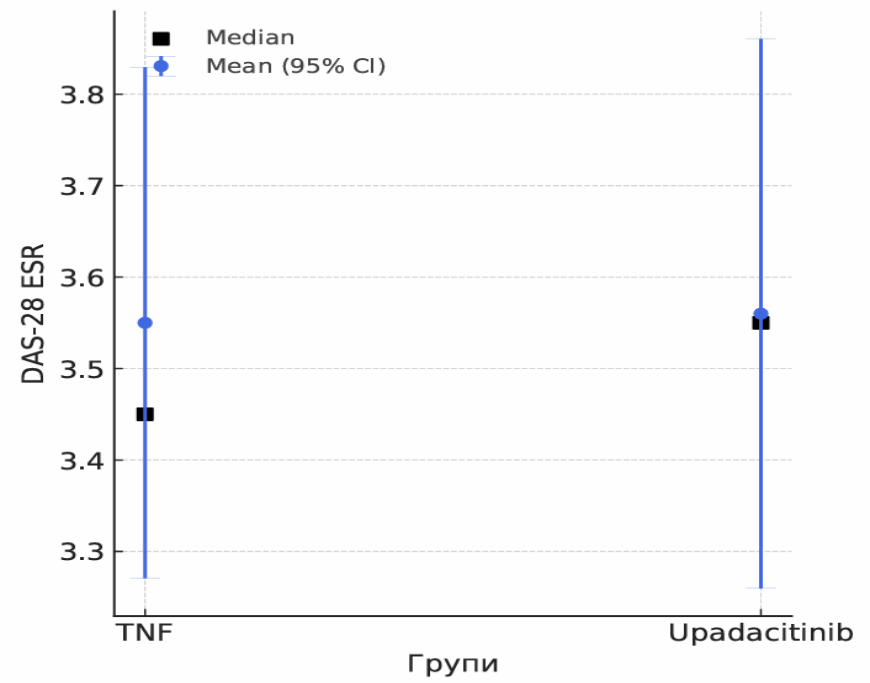
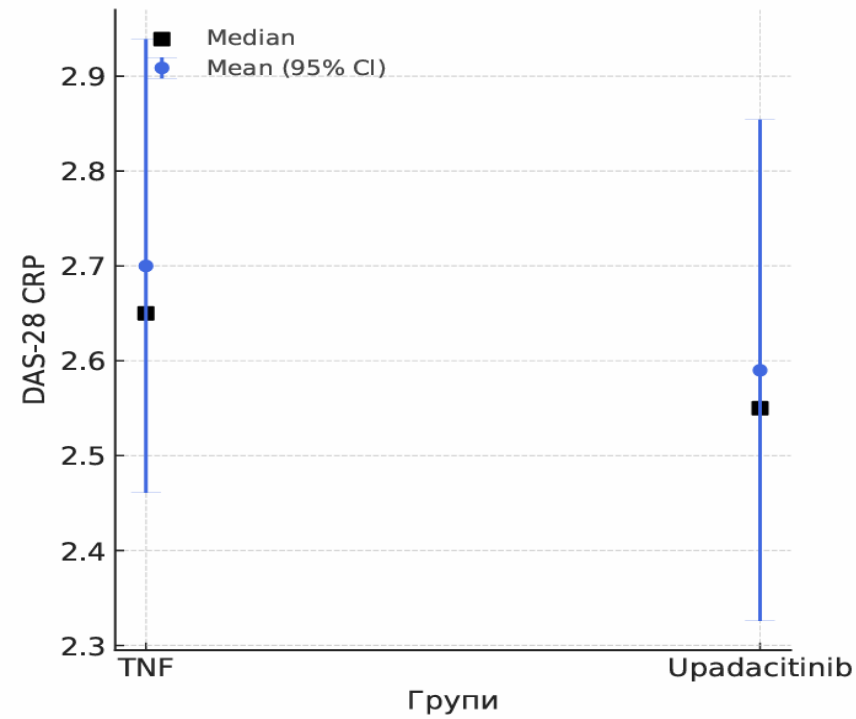


Figure 20. Comparison of DAS28-ESR between the study groups.



Фиг. 21. Сравнение спрямо DAS28-CRP

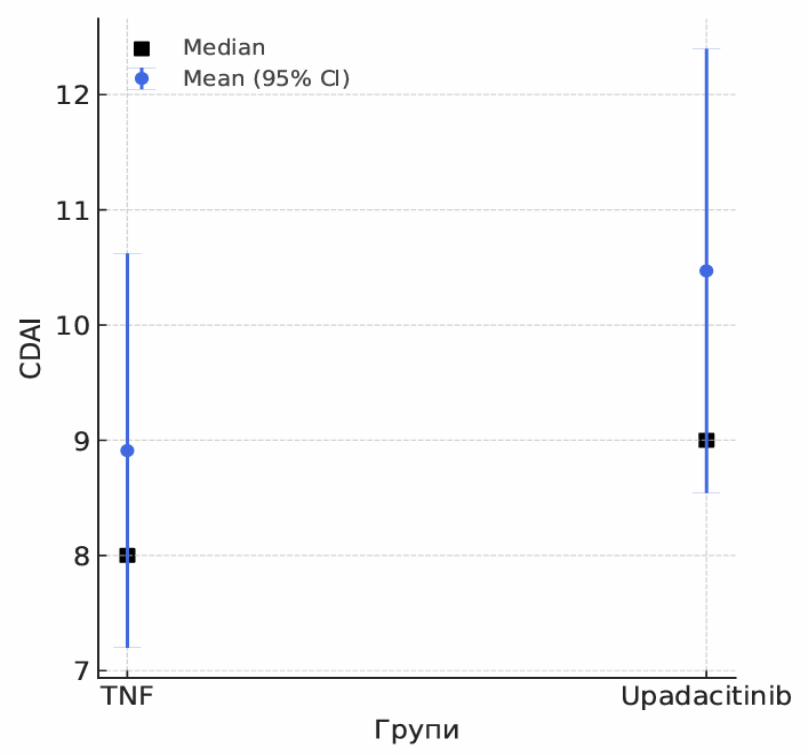


Figure 21. Comparison of DAS28-CRP between the study groups.

2.5. Ultrasound Markers of Arterial Stiffness and ADMA

The analysis of ultrasound-derived vascular parameters among the three study groups—patients treated with TNF inhibitors (n = 41), patients treated with upadacitinib (n = 38), and healthy controls (n = 30)—using one-way analysis of variance (ANOVA) revealed statistically significant differences in two parameters: arterial compliance (AC) and elastic modulus (Ep).

For arterial compliance, a significant group effect was observed ($F = 3.888$, $df = 2$, $p = 0.023$). Tukey post hoc analysis demonstrated a statistically significant difference between the upadacitinib group and the control group (mean difference = −0.208, $p = 0.020$), whereas the differences between the TNF inhibitor group and the other groups did not reach statistical significance (TNF vs. upadacitinib: $p = 0.710$; TNF vs. control: $p = 0.110$).

Similarly, a significant difference was identified for the elastic modulus (Ep) ($F = 4.331$, $df = 2$, $p = 0.017$). The Games–Howell post hoc test revealed a significant difference between the upadacitinib group and the control group (mean difference = 36.1, $p = 0.017$), while no significant differences were detected between the TNF inhibitor group and the remaining groups (TNF vs. upadacitinib: $p = 0.529$; TNF vs. control: $p = 0.340$).

Although the ANOVA for pulse wave velocity (PWV) did not reach statistical significance ($F = 2.594$, $df = 2$, $p = 0.070$), the Tukey post hoc analysis demonstrated a borderline difference between the upadacitinib group and the control group (mean difference = 0.932, $p = 0.063$), which may be interpreted as a trend. No statistically significant differences were observed between the remaining group comparisons (TNF vs. upadacitinib: $p = 0.552$; TNF vs. control: $p = 0.378$).

For the remaining parameters—augmentation index (AI; $F = 2.096$, $df = 2$, $p = 0.131$), beta-stiffness index ($F = 1.446$, $df = 2$, $p = 0.240$), and ADMA concentrations ($F = 0.432$, $df = 2$, $p = 0.651$)—no statistically significant differences were identified among the three study groups.

Table 21. Comparison of arterial stiffness parameters among the study groups (ANOVA test). Note: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	Group	N	Mean	Standard Deviation	ANOVA F / p
PWV (m/s)	TNF	41	6.417	2.013	F = 2.594, df = 2, p = 0.070
	Upadacitinib	38	6.811	1.726	
	Control	30	5.878	0.936	
AI (%)	TNF	41	19.390	14.249	F = 2.096, df = 2, p = 0.131
	Upadacitinib	38	25.574	18.979	
	Control	30	18.333	9.694	

β-stiffness	TNF	41	8.720	7.232	F = 1.446, df = 2, p = 0.240
	Upadacitinib	38	9.511	4.482	
	Control	30	7.280	2.973	
AC (mm/kPa)	TNF	41	0.708	0.273	F = 3.888, df = 2, p = 0.023
	Upadacitinib	38	0.652	0.319	
	Control	30	0.861	0.354	
EP (kPa)	TNF	41	113.024	74.803	F = 4.331, df = 2, p = 0.017
	Upadacitinib	38	130.421	68.377	
	Control	30	94.367	33.450	
ADMA (µmol/L)	TNF	41	0.795	0.256	F = 0.432, df = 2, p = 0.651
	Upadacitinib	38	0.744	0.214	
	Control	30	0.772	0.262	

Table 22. Tukey Post Hoc Test for Comparison of Arterial Compliance (AC, mm/kPa) between the Study Groups Note: p < 0.05, p < 0.01, p < 0.001.

	TNF	Upadacitinib	Control
TNF		Mean diff. = 0.0557 p = 0.710	Mean diff. = -0.153 p = 0.110
Upadacitinib			Mean diff. = -0.208* p = 0.020
Control			

Табл. 23. Tukey Post-Hoc тест за сравнение на скоростта на пулсовата вълна (PWV, m/s) между групите. Забележка: *p* <.05, **p** <.01, ***p <.001.***

Comparison	***Mean Difference***	***p-value***
TNF vs. Upadacitinib	***0.0557***	***0.710***
TNF vs. Control	***–0.153***	***0.110***
Upadacitinib vs. Control	***–0.208****	***0.020***

Table 24. Games–Howell Post Hoc Test for Comparison of the Augmentation Index (AI, %) between the Study Groups Note: p < 0.05, p < 0.01, p < 0.001.

Comparison	***Mean Difference***	***p-value***
TNF vs. Upadacitinib	***−6.18***	***0.241***
TNF vs. Control	***1.06***	***0.927***

Comparison	*Mean Difference*	*p-value*
Upadacitinib vs. Control	*7.24*	*0.112*

Table 25. Tukey Post Hoc Test for Comparison of the Beta Stiffness Index (β-stiffness) between the Study Groups

Comparison	*Mean Difference*	*p-value*
TNF vs. Upadacitinib	*–0.791*	*0.793*
TNF vs. Control	*1.44*	*0.510*
Upadacitinib vs. Control	*2.23*	*0.213*

Table 26. Games–Howell post hoc test for comparison of the elastic modulus (EP, kPa) between groups. Note: p < .05, p < .01, p < .001.

	TNF	Upadacitinib	Control
TNF		Mean diff. = -17.4 p = 0.529	Mean diff. = 18.7 p = 0.340
Upadacitinib			Mean diff. = 36.1* p = 0.017
Control			

Table 27. Tukey post hoc test for comparison of ADMA levels (µmol/L) between groups. Note: p < .05, p < .01, p < .001.

	TNF	Upadacitinib	Control
TNF		Mean diff. = 0.0510 p = 0.624	Mean diff. = 0.0227 p = 0.921
Upadacitinib			Mean diff. = -0.0283 p = 0.883
Control			

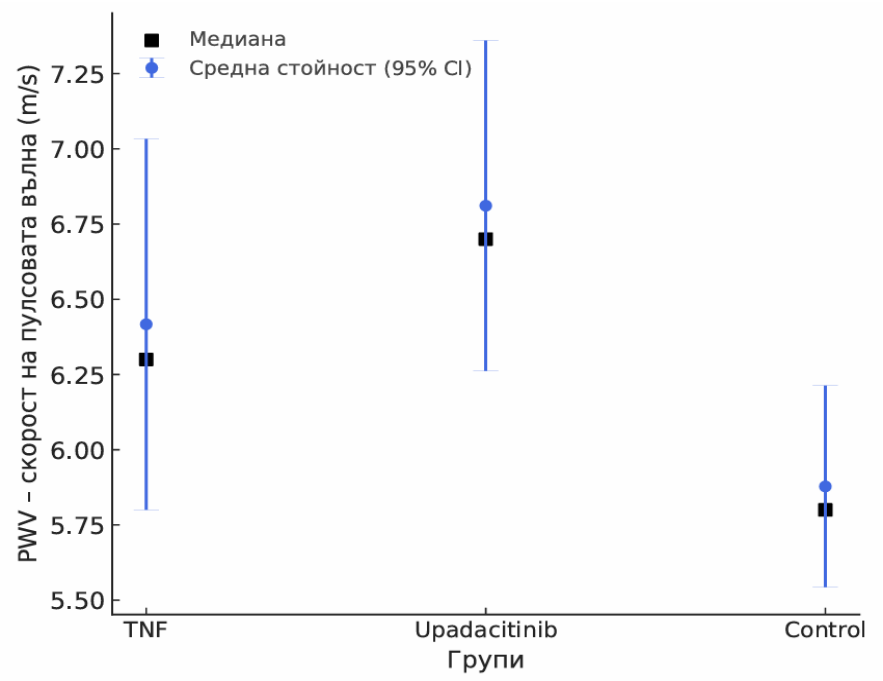


Figure 23. Comparison of groups according to PWV.

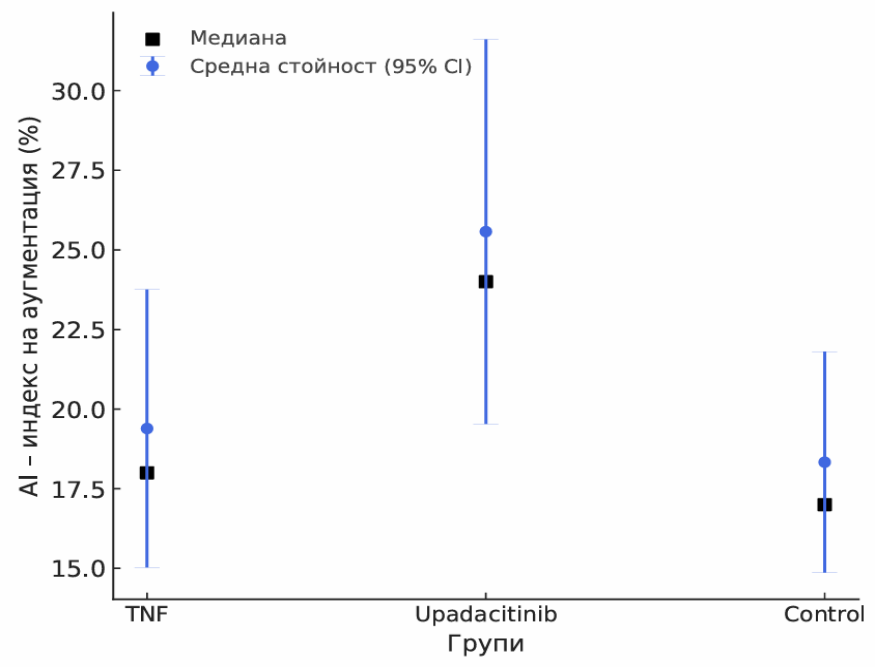


Figure 24. Comparison of groups according to AI.

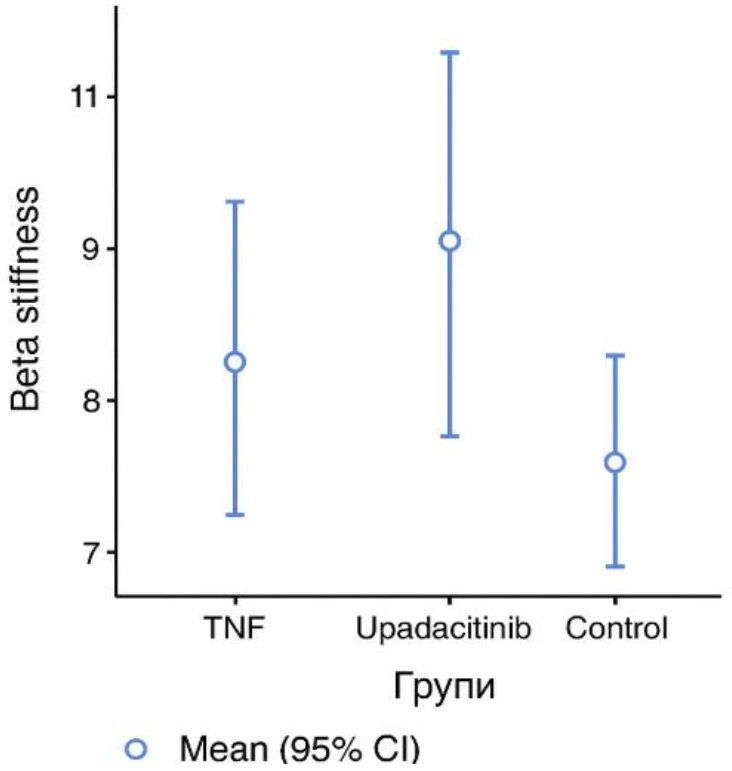


Figure 25. Comparison of groups according to β-stiffness index.

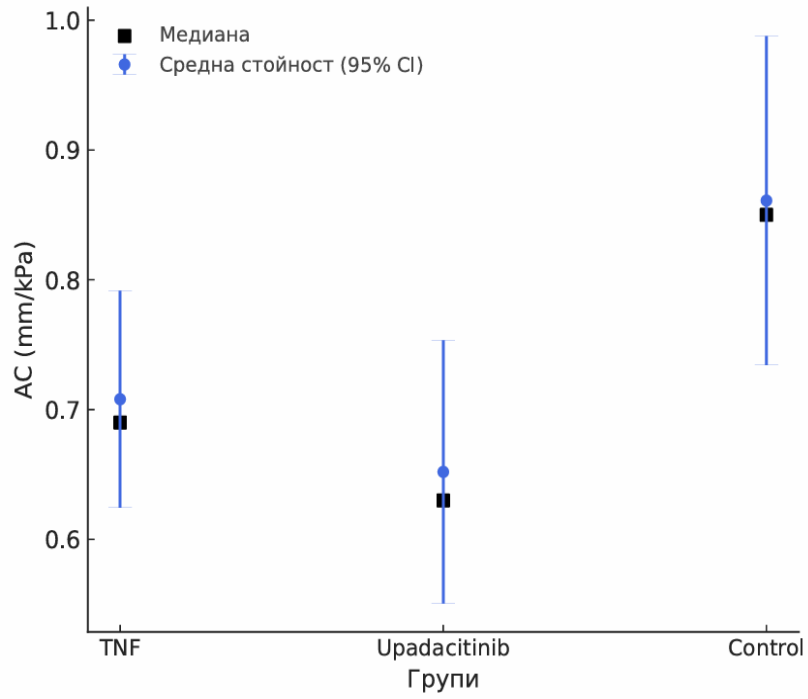


Figure 26. Comparison of groups according to arterial compliance (AC).

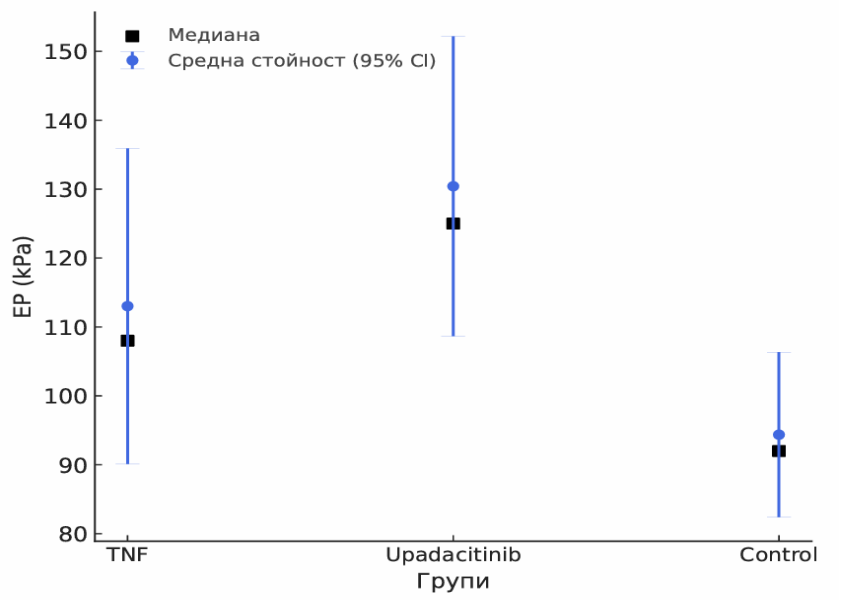


Figure 27. Comparison of groups according to the elastic modulus (EP).

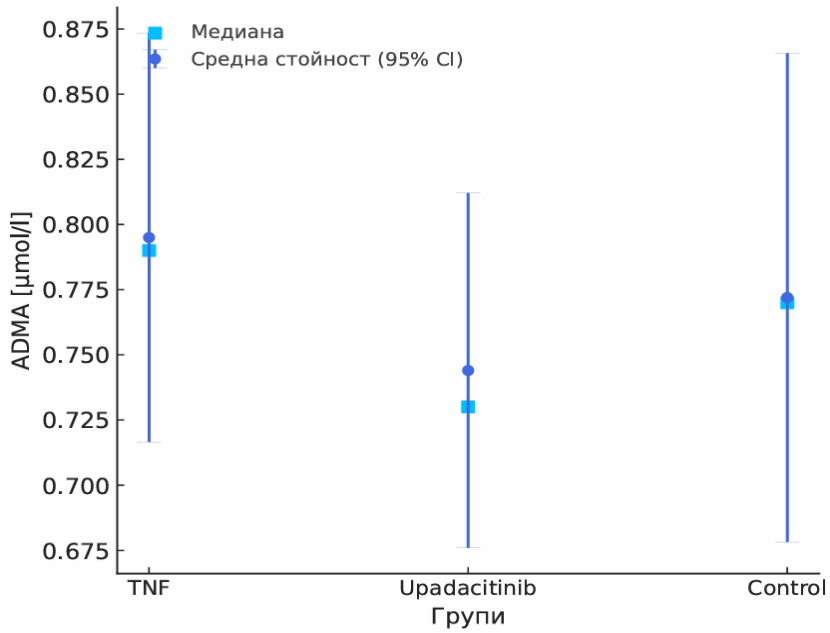


Figure 28. Comparison of groups according to ADMA levels.

3. Correlation analysis of the TNF inhibitor–treated group

In the TNF inhibitor–treated group, significant associations were observed between age and markers of arterial stiffness. Increasing age was associated with higher pulse wave velocity (PWV; r = 0.429, p = 0.005), beta-stiffness index (r = 0.395, p = 0.011), and equivalent elastic

modulus (EP; r = 0.437, p = 0.004), confirming that arterial stiffness increases with advancing age. PWV demonstrated an exceptionally strong positive correlation with the beta-stiffness index (r = 0.951, p < .001) and with EP (r = 0.991, p < .001), indicating that these three parameters assess closely related aspects of vascular stiffness.

At the same time, these stiffness indices were significantly inversely correlated with arterial compliance (AC), with PWV and EP showing negative correlations with AC (r = −0.583 and r = −0.586, respectively; p < .001), and the beta-stiffness index also inversely correlated with AC (r = −0.516, p < .001). These findings confirm that increasing arterial stiffness is associated with a reduced ability of the arteries to distend. A moderate negative correlation was observed between the beta-stiffness index and the augmentation index (AI; r = −0.335, p = 0.032), which may reflect alterations in peripheral vascular resistance.No statistically significant associations were identified between body mass index (BMI) and the other examined parameters (all p > 0.05), suggesting that, in this group, body mass was not a determining factor for vascular function.

Table 28. Correlations between age, BMI, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus.

	BMI	***Age***	***PWV (m/s)***	***AI (%)***	***β-stiffness***	***AC (mm/kPa)***	***EP (kPa)***
BMI	***—***						
Age	***0.242***	***—***					
PWV (m/s)	***0.147***	***0.429*****	***—***				
AI (%)	***−0.149***	***0.064***	***−0.291***	***—***			
β-stiffness	***0.053***	***0.395****	***0.951******	***−0.335****	***—***		
AC (mm/kPa)	***−0.085***	***−0.072***	***−0.583******	***−0.137***	***−0.516******	***—***	
EP (kPa)	***0.136***	***0.437****	***0.991******	***−0.297***	***0.940******	***−0.586******	***—***

In the analysis of the relationship between arterial stiffness parameters and sex (1 = women, 2 = men), a statistically significant positive correlation was observed between sex and arterial compliance (AC) (Spearman's ρ = 0.466, p = 0.002). This finding indicates that men in the studied sample exhibited higher arterial compliance values, reflecting greater arterial elasticity

compared with women. However, this result is difficult to interpret and most likely represents a type I error related to the small sample size of the group. The associations between sex and the remaining arterial stiffness parameters—pulse wave velocity (PWV), augmentation index (AI), β-stiffness index, and elastic modulus (EP)—did not reach statistical significance ($p > 0.05$). With regard to current smoking status, no significant correlations were identified with any of the arterial stiffness markers. All Spearman's ρ values were low (ranging from −0.071 to 0.147), and p values ranged from 0.357 to 0.990, indicating the absence of a statistically significant relationship between smoking and arterial function in the studied group. In contrast, levels of the endothelial dysfunction marker asymmetric dimethylarginine (ADMA) were positively correlated with current smoking (Spearman's $\rho = 0.332$, $p = 0.034$).

Table 29. Correlations between sex, smoking status, and markers of arterial stiffness (Spearman's rho). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; Β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	sex	Smocking-current
Sex	-0.167	-0.216	-0.185	0.466**	-0.140	—	
Df	39	39	39	39	39	—	
p-value	0.298	0.175	0.248	0.002	0.381	—	
Smocking-current	-0.002	0.147	-0.023	-0.071	0.027	-0.064	—
df	39	39	39	39	39	39	—
p-value	0.990	0.357	0.887	0.661	0.867	0.693	—

Table 30. Correlations between sex, smoking status, and ADMA levels (Spearman's rho). Note: p < .05, p < .01, p < .001. Abbreviations: ADMA – asymmetric dimethylarginine.

	sex (1=f, 2=m)	Smocking-current	ADMA (µmol/l)
sex (1=f, 2=m)	—		
Smocking-current	-0.064	—	
ADMA (µmol/l)	-0.135	0.332*	—
df	39	39	—
p-value	0.693	0.034	—

3.1. Previous biologic therapy

In the TNF inhibitor–treated group, correlation analysis with respect to prior use of biologic medications did not identify any statistically significant associations.

Table 31. Correlations between prior biologic therapy and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	Prior biologic therapy	PWV (m/s)	AI (%)	β-stiffness	AC (mm/kPa)	EP (kPa)	ADMA (µmol/L)	Framingham score 10-year risk of MI or death (%)
Prior biologic therapy	—							
PWV (m/s)	0.062	—						
AI (%)	0.185	−0.291	—					
β-stiffness	0.090	0.951***	-0.335*	—				
AC (mm/kPa)	−0.176	-0.583***	−0.137	-0.516***	—			
EP (kPa)	0.043	0.991***	−0.297	0.940***	-0.586***	—		
ADMA (µmol/L)	0.169	0.062	0.094	0.061	−0.074	0.065	—	
Framingham score 10-year risk of MI or death (%)	−0.012	0.257	−0.140	0.234	0.052	0.264	−0.045	—

3.2. Disease duration

In patients receiving TNF inhibitors, disease duration showed a statistically significant positive correlation with pulse wave velocity (PWV) ($r = 0.369$, $p = 0.018$), β-stiffness index ($r = 0.327$, $p = 0.037$), and elastic modulus (EP) ($r = 0.368$, $p = 0.018$), suggesting a progressive worsening of arterial stiffness with increasing disease duration. A moderate negative correlation was also observed with arterial compliance (AC) ($r = −0.429$, $p = 0.005$), further supporting this trend. No statistically significant associations were identified with augmentation index (AI), ADMA levels, or Framingham risk score.

Table 32. Correlations between markers of arterial stiffness, ADMA, Framingham risk, and disease duration (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	PWV (m/s)	AI (%)	β-stiffness	AC (mm/kPa)	EP (kPa)	ADMA (µmol/L)	Framingham score 10-year risk of MI or death (%)	Disease duration (years)
PWV (m/s)	—							
AI (%)	−0.291	—						
β-stiffness	0.951***	−0.335*	—					
AC (mm/kPa)	−0.583***	−0.137	−0.516***	—				
EP (kPa)	0.991***	−0.297	0.940***	−0.586***	—			
ADMA (µmol/L)	0.062	0.094	0.061	−0.074	0.065	—		
Framingham score 10-year risk of MI or death (%)	0.257	−0.140	0.234	0.052	0.264	−0.045	—	
Disease duration (years)	0.369*	−0.009	0.327*	−0.429**	0.368*	−0.030	0.139	—

3.3. Correlations between TNF inhibitor treatment and Framingham Risk Score (FRS)

In the TNF inhibitor–treated group, a significant positive correlation was observed between the Framingham risk of myocardial infarction or death and sex (Spearman's $\rho = 0.416$, $p = 0.007$), indicating a higher Framingham risk among men. No statistically significant correlation was found between smoking status and Framingham risk ($\rho = 0.158$, $p = 0.324$). No other significant associations with the Framingham Risk Score were identified.

Table 33. Correlations between sex, smoking status, and Framingham risk (Spearman's rho). Note: p < .05, p < .01, p < .001. Abbreviations: Framingham score – 10-year risk of myocardial infarction or death according to the Framingham model.

	sex	Smocking-current	Framingham score 10-year risk of MI or death (%)
sex	—		
Smocking-current	-0.064	—	
Framingham score 10-year risk of MI or death (%)	0.416**	0.158	—
df	39	39	—
p-value	0.007	0.324	—

Table 34. Correlations between Framingham risk and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; Framingham score – 10-year risk of myocardial infarction or death.

	Framingham score 10-year risk of MI or death (%)	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA (µmol/l)
Framingham score 10-year risk of MI or death (%)	—						
PWV (m/s)	0.257	—					
AI (%)	-0.140	-0.291	—				
Beta-stiffness	0.234	0.951***	-0.335*	—			
AC (mm/kPa)	0.052	-0.583***	-0.137	-0.516***	—		
EP (kPa)	0.264	0.991***	-0.297	0.940***	-0.586***	—	
ADMA (µmol/l)	-0.045	0.062	0.094	0.061	-0.074	0.065	—

3.4. Correlations with disease activity indices

Following correlation analysis, no statistically significant associations were identified between markers of early vascular damage and the three disease activity indices used (DAS28-ESR, DAS28-CRP, and CDAI).

Table 35. Correlations between DAS28-ESR, Framingham risk, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; Framingham score – 10-year risk of myocardial infarction or death; DAS28-ESR – Disease Activity Score based on erythrocyte sedimentation rate.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA (µmol/l)	Framingham score 10-year risk of MI or death (%)	DAS28ESR
PWV (m/s)	—							
AI (%)	-0.291	—						
Beta-stiffness	0.951***	-0.335*	—					
AC (mm/kPa)	-0.583***	-0.137	-0.516***	—				
EP (kPa)	0.991***	-0.297	0.940***	-0.586***	—			
ADMA (µmol/l)	0.062	0.094	0.061	-0.074	0.065	—		
Framingham score 10-year risk of MI or death (%)	0.257	-0.140	0.234	0.052	0.264	-0.045	—	
DAS28-ESR	0.098	0.164	0.105	-0.030	0.099	0.164	0.104	—

Table 36. Correlations between DAS28-CRP, Framingham risk, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; Framingham score – 10-year risk of myocardial infarction or death; DAS28-CRP – Disease Activity Score based on C-reactive protein levels.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA (µmol/l)	Framingham score 10-year risk of MI or death (%)	DAS28-CRP
PWV (m/s)	—							
AI (%)	-0.291	—						
Beta-stiffness	0.951***	-0.335*	—					
AC (mm/kPa)	-0.583***	-0.137	-0.516***	—				
EP (kPa)	0.991***	-0.297	0.940***	-0.586***	—			
ADMA (µmol/l)	0.062	0.094	0.061	-0.074	0.065	—		
Framingham score 10-year risk of MI or death (%)	0.257	-0.140	0.234	0.052	0.264	-0.045	—	
DAS28-CRP	-0.055	0.030	-0.031	0.095	-0.069	0.027	-0.090	—

Table 37. Correlations between CDAI, Framingham risk, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; CDAI – Clinical Disease Activity Index.

	PWV- m/s	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA	Framingham score 10-year risk of MI or death for this patient in %	CDAI
PWV- m/s	—							
AI- %	-0.291	—						
Beta-stiffness	0.951***	-0.335*	—					
AC- mm/kPa	-0.583***	-0.137	-0.516***	—				
EP- kPa	0.991***	-0.297	0.940***	-0.586***	—			
ADMA (µmol/l)	0.062	0.094	0.061	-0.074	0.065	—		
Framingham score 10-year risk of MI or death for this patient in %	0.257	-0.140	0.234	0.052	0.264	-0.045	—	
CDAI	0.095	0.160	0.115	-0.170	0.090	0.078	-0.054	—

3.5. Analysis of disease activity thresholds

An analysis was also conducted to evaluate the effect of disease activity thresholds on markers of vascular damage. One-way ANOVA did not demonstrate statistically significant differences among disease activity categories defined by the various indices, despite a numerical trend toward deterioration of vascular function at higher levels of disease activity.

Table 38. Arterial stiffness parameters and markers according to DAS28-ESR disease activity categories (ANOVA).Note: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	*DAS28-ESR category*	*N*	*Mean*	*SD*	*95% CI (Lower)*	*95% CI (Upper)*	*Min*	*Max*	*ANOVA (F / p)*
PWV (m/s)	*Remission*	*5*	*5.62*	*1.03*	*4.34*	*6.89*	*4.50*	*6.80*	*F = 0.34, p = 0.80*
	Low activity	*10*	*6.37*	*1.30*	*5.44*	*7.30*	*5.10*	*9.50*	
	Moderate activity	*25*	*6.61*	*2.40*	*5.62*	*7.60*	*4.30*	*15.70*	
	High activity	*1*	*6.00*	*—*	*—*	*—*	*6.00*	*6.00*	
	Total	*41*	*6.42*	*2.01*	*5.78*	*7.05*	*4.30*	*15.70*	
AI (%)	*Remission*	*5*	*10.86*	*11.20*	*−3.05*	*24.77*	*−4.30*	*20.60*	*F = 0.77, p = 0.52*
	Low activity	*10*	*20.77*	*9.27*	*14.14*	*27.40*	*4.90*	*39.60*	

	Moderate activity	*25*	*20.20*	*16.29*	*13.47*	*26.92*	*−16.40*	*52.10*	
	High activity	*1*	*28.10*	*—*	*—*	*—*	*28.10*	*28.10*	
	Total	*41*	*19.39*	*14.25*	*14.89*	*23.89*	*−16.40*	*52.10*	
β-stiffness	*Remission*	*5*	*6.84*	*2.61*	*3.60*	*10.08*	*3.90*	*9.30*	*F = 0.32, p = 0.81*
	Low activity	*10*	*7.61*	*1.71*	*6.39*	*8.83*	*5.10*	*9.50*	
	Moderate activity	*25*	*9.60*	*9.10*	*5.85*	*13.36*	*3.60*	*48.00*	
	High activity	*1*	*7.10*	*—*	*—*	*—*	*7.10*	*7.10*	
	Total	*41*	*8.72*	*7.23*	*6.44*	*11.00*	*3.60*	*48.00*	
AC (mm/kPa)	*Remission*	*5*	*0.72*	*0.20*	*0.47*	*0.96*	*0.56*	*0.97*	*F = 1.27, p = 0.30*
	Low activity	*10*	*0.81*	*0.32*	*0.58*	*1.03*	*0.32*	*1.46*	
	Moderate activity	*25*	*0.65*	*0.26*	*0.55*	*0.76*	*0.16*	*1.30*	
	High activity	*1*	*1.04*	*—*	*—*	*—*	*1.04*	*1.04*	
	Total	*41*	*0.71*	*0.27*	*0.62*	*0.79*	*0.16*	*1.46*	
EP (kPa)	*Remission*	*5*	*85.40*	*31.17*	*46.70*	*124.10*	*51*	*119*	*F = 0.36, p = 0.78*
	Low activity	*10*	*107.40*	*50.53*	*71.25*	*143.55*	*53*	*234*	
	Moderate activity	*25*	*121.60*	*89.11*	*84.82*	*158.38*	*48*	*440*	
	High activity	*1*	*93.00*	*—*	*—*	*—*	*93*	*93*	
	Total	*41*	*113.02*	*74.80*	*89.41*	*136.64*	*48*	*440*	
ADMA (μmol/L)	*Remission*	*5*	*0.79*	*0.35*	*0.35*	*1.22*	*0.38*	*1.30*	*F = 0.10, p = 0.82*
	Low activity	*10*	*0.75*	*0.33*	*0.52*	*0.98*	*0.34*	*1.38*	
	Moderate activity	*25*	*0.81*	*0.21*	*0.72*	*0.90*	*0.37*	*1.29*	
	High activity	*1*	*0.99*	*—*	*—*	*—*	*0.99*	*0.99*	
	Total	*41*	*0.79*	*0.26*	*0.71*	*0.88*	*0.34*	*1.38*	

Табл. 39. Table 39. Association between arterial stiffness markers and DAS28-CRP disease activity categories.Note: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	DAS28-CRP category	N	Mean	SD	95% CI	Min	Max	ANOVA (F, p)
PWV (m/s)	Remission	20	6.68	2.47	5.53–7.84	4.50	15.70	F = 0.48, p = 0.62

	Low activity	11	6.39	1.76	5.21–7.57	5.00	10.90	
	Moderate activity	10	5.91	1.10	5.12–6.70	4.30	8.50	
AI (%)	Remission	20	20.01	14.39	13.28–26.74	−7.8	52.1	F = 0.04, p = 0.96
	Low activity	11	18.97	14.02	9.55–28.39	0.3	51.2	
	Moderate activity	10	18.61	15.66	7.41–29.81	−16.4	33.3	
β-stiffness	Remission	20	9.65	9.41	5.24–14.05	3.9	48.0	F = 0.42, p = 0.66
	Low activity	11	8.55	5.44	4.89–12.20	4.9	23.3	
	Moderate activity	10	7.06	2.75	5.09–9.03	3.6	14.0	
AC (mm/kPa)	Remission	20	0.68	0.30	0.54–0.82	0.16	1.46	F = 0.39, p = 0.68
	Low activity	11	0.77	0.29	0.58–0.96	0.30	1.30	
	Moderate activity	10	0.69	0.21	0.54–0.84	0.33	1.04	
EP (kPa)	Remission	20	122.50	88.02	81.30–163.70	51	440	F = 0.58, p = 0.57
	Low activity	11	115.55	75.27	64.98–166.11	64	320	
	Moderate activity	10	91.30	38.53	63.74–118.86	48	186	
ADMA (µmol/L)	Remission	20	0.83	0.23	0.72–0.93	0.35	1.30	F = 0.40, p = 0.67
	Low activity	11	0.74	0.27	0.56–0.92	0.38	1.38	
	Moderate activity	10	0.80	0.30	0.58–1.01	0.34	1.29	

Table 40. Association between arterial stiffness markers and CDAI disease activity categories. Note: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	CDAI category	N	Mean	SD	95% CI	Min	Max	ANOVA (F, p)
PWV (m/s)	Remission	6	5.55	0.93	4.57–6.53	4.50	6.80	F = 0.64, p = 0.59
	Low activity	19	6.35	1.54	5.61–7.09	4.70	10.90	
	Moderate activity	15	6.88	2.77	5.35–8.41	4.30	15.70	
	High activity	1	6.00	—	—	6.00	6.00	
AI (%)	Remission	6	11.50	10.14	0.86–22.15	−4.3	20.6	F = 0.83, p = 0.49
	Low activity	19	21.13	9.77	16.42–25.84	4.10	39.60	
	Moderate activity	15	19.76	19.60	8.90–30.62	−16.4	52.10	
	High activity	1	28.10	—	—	28.10	28.10	
β-stiffness	Remission	6	6.60	2.41	4.07–9.13	3.90	9.30	F = 0.53, p = 0.66
	Low activity	19	8.05	4.05	6.10–10.01	4.40	23.30	
	Moderate activity	15	10.52	10.96	4.45–16.59	3.60	48.00	

	High activity	1	7.10	—	—	7.10	7.10	
AC (mm/kPa)	Remission	6	0.84	0.35	0.47–1.21	0.56	1.46	F = 1.63, p = 0.20
	Low activity	19	0.72	0.25	0.60–0.84	0.30	1.30	
	Moderate activity	15	0.62	0.26	0.47–0.76	0.16	1.03	
	High activity	1	1.04	—	—	1.04	1.04	
EP (kPa)	Remission	6	83.00	28.49	53.10–112.90	51	119	F = 0.57, p = 0.64
	Low activity	19	110.74	64.15	79.82–141.66	53	320	
	Moderate activity	15	129.27	98.49	74.72–183.81	48	440	
	High activity	1	93.00	—	—	93	93	
ADMA (µmol/L)	Remission	6	0.76	0.32	0.43–1.10	0.38	1.30	F = 0.23, p = 0.88
	Low activity	19	0.80	0.28	0.67–0.93	0.34	1.38	
	Moderate activity	15	0.79	0.22	0.67–0.91	0.37	1.29	
	High activity	1	0.99	—	—	0.99	0.99	

4. Correlation analysis of the group treated with the JAK inhibitor upadacitinib

In the upadacitinib-treated group, strong and statistically significant associations were observed between age and markers of arterial stiffness. Higher age was associated with a significant increase in pulse wave velocity (PWV; $r = 0.579$, $p < .001$), β-stiffness index ($r = 0.540$, $p < .001$), and equivalent elastic modulus (EP; $r = 0.565$, $p < .001$), as well as with a significant decrease in arterial compliance (AC; $r = −0.450$, $p = 0.005$). Similar to the findings in the TNF inhibitor–treated group, PWV exhibited very strong positive correlations with the β-stiffness index ($r = 0.981$, $p < .001$) and with EP ($r = 0.983$, $p < .001$), indicating a high degree of concordance among these indices of vascular stiffness. At the same time, PWV, β-stiffness, and EP were significantly inversely correlated with AC ($r = −0.733$, $r = −0.678$, and $r = −0.683$, respectively; all $p < .001$), reflecting reduced arterial elasticity with increasing vascular rigidity. The augmentation index (AI) did not show significant associations with any of the other parameters (all $p > 0.05$). Body mass index (BMI) was likewise not significantly associated with arterial parameters (all $p > 0.05$), suggesting that BMI was not a determining factor for vascular function in this group.

Table 41. Correlations between age, BMI, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	BMI	Age	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)
BMI	—						
Age	0.128	—					
PWV (m/s)	0.209	0.579***	—				
AI (%)	0.265	0.016	0.013	—			
Beta-stiffness	0.149	0.540***	0.981***	0.006	—		
AC (mm/kPa)	-0.230	-0.450**	-0.733***	0.011	-0.678***	—	
EP (kPa)	0.145	0.565***	0.983***	0.020	0.988***	-0.683***	—

4.1. Sex and smoking status

In the analysis of the relationship between arterial stiffness parameters and sex (1 = women, 2 = men), no statistically significant correlations were identified with the measured variables in this group. Similarly, no significant associations were observed between smoking status and the ultrasound-derived markers of arterial stiffness.

In contrast, a statistically significant positive correlation was found between ADMA levels and smoking status as well as male sex (Spearman's $\rho = 0.362$, $p = 0.025$; and Spearman's $\rho = 0.477$, $p = 0.002$, respectively). These findings indicate that the serological marker of endothelial dysfunction is influenced by sex and smoking in patients treated with upadacitinib. Smokers and male patients receiving this therapy exhibit impaired endothelial function.

Table 42. Correlations between sex, smoking status, and markers of arterial stiffness (Spearman's rho). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	sex	Smocking-current
sex	0.199	0.149	0.180	-0.110	0.180	—	
df	36	36	36	36	36	—	
p-value	0.230	0.373	0.280	0.513	0.280	—	
Smocking-current	0.031	0.103	0.012	-0.026	0.034	0.343*	—
df	36	36	36	36	36	36	—
p-value	0.852	0.537	0.943	0.875	0.841	0.035	—

Table 43. Correlations between ADMA, sex, and smoking status (Spearman's rho). Note: p < .05, p < .01, p < .001. Abbreviations: ADMA – asymmetric dimethylarginine.

	ADMA (µmol/l)	sex 1=f, 2=m	Smocking-current
ADMA (µmol/l)	—		
sex 1=f, 2=m	ρ = 0.477**	—	
df	36	—	
p-value	0.002	—	
Smocking-current	ρ = 0.362*	ρ = 0.343*	—
df	36	36	—
p-value	0.025	0.035	—

4.2. Prior biologic therapy

In the upadacitinib-treated group, correlation analysis with respect to prior biologic therapy and disease duration did not reveal any statistically significant associations.

Table 44. Correlations between prior biologic therapy, disease duration, and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	Disease duration (years)	Previous biologics	ADMA (µmol/l)	EP (kPa)	AC (mm/kPa)	Beta-stiffness	AI (%)	PWV (m/s)
Disease duration (years)	—	0.520***	0.025	-0.184	0.196	-0.180	-0.061	-0.191
Previous biologics	0.520***	—	-0.112	0.012	0.171	-0.005	-0.177	0.022
ADMA (µmol/l)	0.025	-0.112	—	0.163	0.054	0.137	0.269	0.151
EP (kPa)	-0.184	0.012	0.163	—	-0.755***	0.989***	0.092	0.995***
AC (mm/kPa)	0.196	0.171	0.054	-0.755***	—	-0.744***	0.004	-0.741***
Beta-stiffness	-0.180	-0.005	0.137	0.989***	-0.744***	—	0.089	0.992***
AI (%)	-0.061	-0.177	0.269	0.092	0.004	0.089	—	0.070
PWV (m/s)	-0.191	0.022	0.151	0.995***	-0.741***	0.992***	0.070	—

4.3. Correlations with the Framingham Risk Score (FRS)

Results from the Pearson correlation analysis demonstrated strong positive associations between the Framingham risk of myocardial infarction or death and markers of arterial stiffness. A significant correlation was observed between FRS and pulse wave velocity (PWV) ($r = 0.548$, $p < .001$), indicating greater arterial stiffness in individuals with higher cardiovascular risk. Pearson correlation analysis also revealed a strong positive association between FRS and the β-stiffness index ($r = 0.566$, $p < .001$), as well as between FRS and the elastic modulus (EP) ($r = 0.573$, $p < .001$). These findings suggest that an increased Framingham risk is associated with higher EP values, reflecting impaired arterial elasticity.

Table 45. Correlations between Framingham risk and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; MI – myocardial infarction.

	Framingham score 10-year risk of MI or death (%)	ADMA (µmol/l)	EP (kPa)	AC (mm/kPa)	Beta-stiffness	AI (%)	PWV (m/s)
Framingham score 10-year risk of MI or death (%)	—	0.148	0.573***	-0.317	0.566***	0.190	0.548***
ADMA (µmol/l)		—	0.152	-0.090	0.153	0.317	0.175
EP (kPa)			—	-0.683***	0.988***	0.020	0.983***
AC (mm/kPa)				—	-0.678***	0.011	-0.733***
Beta-stiffness					—	0.006	0.981***
AI (%)						—	0.013
PWV (m/s)							—

4.4. Correlations with disease activity indices

Following correlation analysis, a statistically significant negative association was identified between arterial compliance and the Clinical Disease Activity Index (CDAI). This finding indicates that higher CDAI values are associated with reduced arterial compliance and increased arterial stiffness. No other statistically significant correlations were observed between the remaining markers of early vascular damage and the three disease activity indices used (DAS28-ESR, DAS28-CRP, and CDAI).

Table 46. Correlations between DAS28-ESR and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – beta-stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine; Framingham score – Framingham risk score.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA	Framingham score	DAS28-ESR
PWV (m/s)	—							
AI (%)	0.013	—						
Beta-stiffness	0.981***	0.006	—					
AC (mm/kPa)	-0.733***	0.011	-0.678***	—				
EP (kPa)	0.983***	0.020	0.988***	-0.683***	—			
ADMA (µmol/l)	0.175	0.317	0.153	-0.090	0.152	—		
Framingham score	0.548***	0.190	0.566***	-0.317	0.573***	0.148	—	
DAS28-ESR	0.265	-0.004	0.241	-0.233	0.201	-0.097	-0.111	—

Table 47. Correlations between DAS28-CRP and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – pulse wave velocity; AI – augmentation index; β-stiffness – arterial stiffness index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA	Framingham score 10-year risk of MI or death for this patient in %	DAS28-CRP
PWV- m/s	—							
AI- %	0.013	—						
Beta-stiffness	0.981***	0.006	—					
AC- mm/kPa	-0.733***	0.011	-0.678***	—				
EP- kPa	0.983***	0.020	0.988***	-0.683***	—			
ADMA (µmol/l)	0.175	0.317	0.153	-0.090	0.152	—		
Framingham score 10-year risk of MI or death for this patient in %	0.548***	0.190	0.566***	-0.317	0.573***	0.148	—	
DAS28-CRP	0.130	0.036	0.078	-0.277	0.054	-0.042	-0.211	—

Table 48. Correlations between CDAI and markers of arterial stiffness (Pearson's r). Note: p < .05, p < .01, p < .001. Abbreviations: PWV – Pulse Wave Velocity; AI – Augmentation Index; AC – Arterial Compliance; EP – Elastic Modulus; ADMA – Asymmetric Dimethylarginine.

	PWV (m/s)	AI (%)	Beta-stiffness	AC (mm/kPa)	EP (kPa)	ADMA	Framingham score 10-year risk of MI or death for this patient in %	CDAI
PWV (m/s)	—							
AI (%)	0.013	—						
Beta-stiffness	0.981***	0.006	—					
AC (mm/kPa)	-0.733***	0.011	-0.678***	—				
EP (kPa)	0.983***	0.020	0.988***	-0.683***	—			
ADMA(µmol/l)	0.175	0.317	0.153	-0.090	0.152	—		
Framingham score 10-year risk of MI or death for this patient in %	0.548***	0.190	0.566***	-0.317	0.573***	0.148	—	
CDAI	0.314	0.071	0.273	-0.328*	0.249	0.054	-0.121	—

4.5. Analysis of disease activity thresholds

Analysis according to disease activity thresholds did not reveal any statistically significant associations with markers of endothelial dysfunction or arterial stiffness.

Table 49. Arterial stiffness parameters and vascular markers according to DAS28-ESR disease activity categories (ANOVA). Note: PWV – pulse wave velocity; AI – augmentation index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	DAS28-ESR category	N	Mean	SD	95% CI (Lower)	95% CI (Upper)	Min	Max	ANOVA (F, p)
PWV (m/s)	Remission	8	5.73	1.49	4.48	6.97	3.5	8.4	F = 1.904, p = 0.148
	Low activity	4	7.05	3.17	2.00	12.10	5.3	11.8	
	Moderate activity	24	7.00	1.45	6.39	7.61	4.2	9.5	
	High activity	2	8.45	0.78	1.46	15.44	7.9	9.0	
	Total	38	6.81	1.73	6.24	7.38	3.5	11.8	
AI (%)	Remission	8	21.06	15.39	8.19	33.93	2.5	48.7	F = 2.256, p = 0.100
	Low activity	4	47.63	24.79	8.18	87.07	28.7	82.0	
	Moderate activity	24	23.58	17.93	16.01	31.15	−21.4	45.3	
	High activity	2	23.40	14.85	−110.02	156.82	12.9	33.9	
	Total	38	25.57	18.98	19.34	31.81	−21.4	82.0	
β-stiffness	Remission	8	6.83	2.97	4.35	9.31	3.3	12.5	F = 1.547, p = 0.220
	Low activity	4	10.28	9.28	−4.50	25.05	5.4	24.2	
	Moderate activity	24	9.99	3.76	8.40	11.58	3.5	17.5	
	High activity	2	12.95	2.90	−13.10	38.99	10.9	15.0	
	Total	38	9.51	4.48	8.04	10.98	3.3	24.2	
AC (mm/kPa)	Remission	8	0.86	0.43	0.50	1.22	0.27	1.69	F = 1.665, p = 0.193
	Low activity	4	0.60	0.20	0.29	0.92	0.33	0.80	
	Moderate activity	24	0.61	0.29	0.49	0.73	0.16	1.30	
	High activity	2	0.47	0.06	−0.04	0.98	0.43	0.51	
	Total	38	0.65	0.32	0.55	0.76	0.16	1.69	
EP (kPa)	Remission	8	91.38	50.14	49.46	133.29	32	195	F = 1.482, p = 0.237
	Low activity	4	148.75	144.23	−80.74	378.24	72	365	
	Moderate activity	24	135.92	55.81	112.35	159.48	46	260	
	High activity	2	184.00	36.77	−146.36	514.36	158	210	
	Total	38	130.42	68.38	107.95	152.90	32	365	
ADMA (µmol/L)	Remission	8	0.77	0.14	0.65	0.88	0.54	0.92	F = 0.561, p = 0.644
	Low activity	4	0.86	0.18	0.58	1.14	0.63	1.05	
	Moderate activity	24	0.71	0.25	0.61	0.82	0.27	1.46	
	High activity	2	0.78	0.01	0.72	0.83	0.77	0.78	
	Total	38	0.74	0.21	0.67	0.81	0.27	1.46	

Table 50. Arterial stiffness parameters and vascular markers according to DAS28-ESR disease activity categories (ANOVA).Note: PWV – pulse wave velocity; AI – augmentation index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	**DAS28-ESR category**	**N**	**Mean**	**SD**	**95% CI (Lower–Upper)**	**Min**	**Max**	**ANOVA (F, p)**
PWV (m/s)	Remission	24	6.67	1.98	5.83–7.51	3.5	11.8	F = 0.664, p = 0.580
	Low activity	8	6.71	1.26	5.66–7.77	5.1	8.4	
	Moderate activity	5	7.20	0.84	6.16–8.24	6.2	8.1	
	High activity	1	9.00	—	—	9.0	9.0	

	Total	38	6.81	1.73	6.24–7.38	3.5	11.8	
AI (%)	Remission	24	23.74	20.86	14.93–32.55	−21.4	82.0	F = 0.484, p = 0.696
	Low activity	8	28.26	16.83	14.20–42.33	4.7	48.7	
	Moderate activity	5	32.60	14.04	15.16–50.04	8.6	45.3	
	High activity	1	12.90	—	—	12.9	12.9	
	Total	38	25.57	18.98	19.34–31.81	−21.4	82.0	
β-stiffness	Remission	24	9.29	5.18	7.10–11.48	3.3	24.2	F = 0.582, p = 0.631
	Low activity	8	9.00	3.11	6.40–11.60	5.1	13.0	
	Moderate activity	5	10.28	2.30	7.42–13.14	7.5	12.9	
	High activity	1	15.00	—	—	15.0	15.0	
	Total	38	9.51	4.48	8.04–10.98	3.3	24.2	
AC (mm/kPa)	Remission	24	0.71	0.35	0.56–0.86	0.16	1.69	F = 0.986, p = 0.411
	Low activity	8	0.62	0.28	0.39–0.86	0.27	1.10	
	Moderate activity	5	0.45	0.15	0.26–0.65	0.25	0.64	
	High activity	1	0.51	—	—	0.51	0.51	
	Total	38	0.65	0.32	0.55–0.76	0.16	1.69	
EP (kPa)	Remission	24	127.92	79.59	94.31–161.52	32	365	F = 0.491, p = 0.691
	Low activity	8	123.25	46.66	84.24–162.26	67	195	
	Moderate activity	5	138.00	34.94	94.62–181.38	99	184	
	High activity	1	210.00	—	—	210	210	
	Total	38	130.42	68.38	107.95–152.90	32	365	
ADMA (µmol/L)	Remission	24	0.75	0.18	0.68–0.83	0.41	1.05	F = 0.074, p = 0.973
	Low activity	8	0.74	0.37	0.44–1.05	0.27	1.46	
	Moderate activity	5	0.70	0.11	0.57–0.84	0.59	0.85	
	High activity	1	0.78	—	—	0.78	0.78	
	Total	38	0.74	0.21	0.67–0.81	0.27	1.46	

Table 51. Arterial stiffness parameters according to CDAI disease activity categories.
Note: PWV – pulse wave velocity; AI – augmentation index; AC – arterial compliance; EP – elastic modulus; ADMA – asymmetric dimethylarginine.

Parameter	CDAI category	N	Mean	SD	95% CI (Lower–Upper)	Min	Max	ANOVA (F, p)
PWV (m/s)	Low activity	25	6.50	1.90	5.72–7.29	3.50	11.80	F = 1.744, p = 0.190
	Moderate activity	11	7.19	1.15	6.42–7.96	5.30	8.50	
	High activity	2	8.55	0.64	2.83–14.27	8.10	9.00	
	Total	38	6.81	1.73	6.24–7.38	3.50	11.80	
AI (%)	Low activity	25	25.68	20.95	17.04–34.33	−21.40	82.00	F = 0.001, p = 0.999
	Moderate activity	11	25.38	15.76	14.79–35.97	2.00	48.70	
	High activity	2	25.25	17.47	−131.67–182.17	12.90	37.60	
	Total	38	25.57	18.98	19.34–31.81	−21.40	82.00	
β-stiffness	Low activity	25	8.87	4.98	6.82–10.93	3.30	24.20	F = 1.376, p = 0.266
	Moderate activity	11	10.16	3.01	8.13–12.18	5.40	14.40	
	High activity	2	13.95	1.48	0.61–27.29	12.90	15.00	
	Total	38	9.51	4.48	8.04–10.98	3.30	24.20	
AC (mm/kPa)	Low activity	25	0.71	0.35	0.57–0.86	0.16	1.69	F = 1.686, p = 0.200
	Moderate activity	11	0.56	0.23	0.41–0.72	0.27	1.03	
	High activity	2	0.38	0.18	−1.27–2.03	0.25	0.51	
	Total	38	0.65	0.32	0.55–0.76	0.16	1.69	
EP (kPa)	Low activity	25	121.92	77.27	90.03–153.81	32.00	365.00	F = 1.217, p = 0.308

	Moderate activity	11	137.64	42.58	109.03–166.24	72.00	195.00	
	High activity	2	197.00	18.39	31.82–362.18	184.00	210.00	
	Total	38	130.42	68.38	107.95–152.90	32.00	365.00	
ADMA (µmol/L)	Low activity	25	0.74	0.24	0.64–0.84	0.27	1.46	F = 0.127, p = 0.881
	Moderate activity	11	0.75	0.17	0.64–0.86	0.46	1.03	
	High activity	2	0.82	0.05	0.35–1.28	0.78	0.85	
	Total	38	0.74	0.21	0.67–0.81	0.27	1.46	

5. Regression analysis

5.1. Analysis of PWV

In a regression model including lipid profile parameters, age, body mass index, and cardiovascular risk, treatment with upadacitinib was associated with a statistically significant increase in PWV compared with the control group (β = 0.80; p = 0.035). Total cholesterol and age were also positively associated with PWV, whereas higher HDL levels were associated with significantly lower arterial stiffness (β = −1.81; p = 0.005). Interestingly, LDL cholesterol and triglycerides showed an inverse association with PWV, which may reflect the effects of lipid-lowering therapy or other confounding factors. Framingham cardiovascular risk score was likewise associated with increased arterial stiffness (β = 0.11; p = 0.048).

A multiple regression analysis including both treatment groups and incorporating disease duration, treatment modality, and disease activity indices was also performed. The results demonstrated that only the Clinical Disease Activity Index (CDAI) was a statistically significant predictor of arterial stiffness as measured by PWV (β = 0.155; p = 0.039), suggesting that higher disease activity is associated with increased PWV. Differences between patients treated with upadacitinib and those receiving TNF inhibitors did not reach statistical significance after adjustment for other clinical variables (p = 0.555). Treatment duration, steroid dose, and serum inflammatory markers (CRP and ESR) were not independently associated with PWV.

Table 52. Multiple regression model for PWV (m/s) (R = 0.589; R^2 = 0.358[a]). Note: Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	Standard Error	95% CI (Lower)	95% CI (Upper)	*t*	*p*
Intercept[a]	3.967	1.659	0.675	7.259	2.391	0.019*
Treatment group						
TNF inhibitors vs control (1–3)	0.293	0.350	−0.401	0.987	0.837	0.405
Upadacitinib vs control (2–3)	0.797	0.373	0.058	1.536	2.139	0.035*
Total cholesterol	1.368	0.562	0.254	2.483	2.437	0.017*
HDL cholesterol	−1.813	0.625	−3.052	−0.573	−2.902	0.005**

LDL cholesterol	−1.318	0.585	−2.479	−0.157	−2.253	0.027*
Triglycerides	−0.726	0.319	−1.359	−0.092	−2.274	0.025*
Body mass index (BMI)	0.036	0.031	−0.026	0.098	1.139	0.257
Age (years)	0.045	0.015	0.015	0.076	2.943	0.004**
Framingham score (10-year risk of MI or death, %)	0.114	0.057	0.001	0.227	2.006	0.048*
Sex (1 = female, 2 = male)	−1.002	0.567	−2.127	0.123	−1.767	0.080

Table 53. Multiple regression model for pulse wave velocity (PWV, m/s). Note: R = 0.405; R^2 = 0.164[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	95% CI (Lower)	95% CI (Upper)	t	p
Intercept[a]	6.843	1.508	3.835	9.851	4.539	< .001***
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	0.319	0.538	−0.754	1.392	0.594	0.555
Treatment duration (years)	0.093	0.077	−0.060	0.247	1.213	0.230
Disease duration (years)	0.002	0.035	−0.068	0.072	0.056	0.955
Steroid dose (prednisolone equivalent)	−0.023	0.085	−0.193	0.147	−0.267	0.790
Previous biologic therapy	0.144	0.274	−0.401	0.690	0.528	0.599
C-reactive protein (CRP)	0.004	0.020	−0.037	0.044	0.179	0.858
Erythrocyte sedimentation rate (ESR)	0.018	0.014	−0.010	0.046	1.251	0.215
Clinical Disease Activity Index (CDAI)	0.155	0.074	0.008	0.303	2.100	0.039*
DAS28-CRP	−0.943	0.529	−1.999	0.113	−1.782	0.079
DAS28-ESR	−0.124	0.484	−1.090	0.842	−0.256	0.799

5.2. Analysis of AI (%)

In a multiple linear regression analysis with AI (%) as the dependent variable, no statistically significant differences were observed between the main therapeutic groups. Patients treated with upadacitinib showed a trend toward higher AI% values compared with both the control group (β = 6.28; p = 0.118) and the TNF inhibitor–treated group (β = 7.75; p = 0.119); however, these differences did not reach statistical significance. Age and smoking status also demonstrated positive associations with AI%, without achieving statistical significance.

C-reactive protein (CRP) was the only variable identified as a significant independent predictor, with higher CRP levels being associated with lower AI% values (β = −0.45; p = 0.019). This finding is contrary to physiological expectations and suggests the possible influence of confounding factors or the need for more in-depth analysis. Lipid profile parameters, body mass index, cardiovascular risk assessed by the Framingham score, disease duration, steroid dose, and disease activity indices were not significantly associated with arterial stiffness as measured by AI%.

Table 54. Multiple regression model for augmentation index (AI%). Note: R = 0.325; R² = 0.106[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	95% CI (Lower)	95% CI (Upper)	*t*	*p*
Intercept[a]	−3.721	18.044	−39.533	32.092	−0.206	0.837
Treatment group						
TNF inhibitors vs control (1–3)	−0.171	3.751	−7.617	7.274	−0.046	0.964
Upadacitinib vs control (2–3)	6.284	3.988	−1.632	14.199	1.576	0.118
Age (years)	0.252	0.179	−0.103	0.606	1.408	0.162
Body mass index (BMI)	0.412	0.333	−0.249	1.073	1.237	0.219
Framingham score (10-year risk of MI or death, %)	−0.488	0.649	−1.777	0.801	−0.752	0.454
Total cholesterol	1.358	5.986	−10.524	13.239	0.227	0.821
Triglycerides	−3.240	3.409	−10.006	3.526	−0.950	0.344
LDL cholesterol	−1.633	6.232	−14.002	10.737	−0.262	0.794
HDL cholesterol	−0.019	6.728	−13.371	13.335	−0.003	0.998
Sex (1 = female, 2 = male)	0.671	6.090	−11.416	12.759	0.110	0.912
Current smoking	4.621	3.428	−2.183	11.424	1.348	0.181

Table 55. Multiple regression model for augmentation index (AI%). Note: R = 0.370; R² = 0.106[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	*Estimate (β)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Intercept[a]	*6.299*	*13.753*	*−21.144*	*33.743*	*0.458*	*0.648*
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	*7.750*	*4.904*	*−2.036*	*17.535*	*1.580*	*0.119*
Disease duration (years)	*0.140*	*0.321*	*−0.500*	*0.780*	*0.436*	*0.665*
Treatment duration (years)	*−0.233*	*0.703*	*−1.635*	*1.169*	*−0.332*	*0.741*
Steroid dose (prednisolone equivalent)	*−0.199*	*0.778*	*−1.751*	*1.353*	*−0.256*	*0.798*
Previous biologic therapy	*−1.103*	*2.495*	*−6.082*	*3.876*	*−0.442*	*0.660*

Predictor	*Estimate (β)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
C-reactive protein (CRP)	*−0.445*	*0.186*	*−0.816*	*−0.075*	*−2.400*	*0.019**
Erythrocyte sedimentation rate (ESR)	*0.003*	*0.128*	*−0.252*	*0.258*	*0.022*	*0.983*
DAS28-ESR	*2.817*	*4.415*	*−5.993*	*11.627*	*0.638*	*0.526*
DAS28-CRP	*1.745*	*4.827*	*−7.887*	*11.377*	*0.362*	*0.719*
Clinical Disease Activity Index (CDAI)	*0.063*	*0.675*	*−1.285*	*1.410*	*0.093*	*0.926*

5.3. Analysis of the β-stiffness index

In a multiple linear regression analysis with the β-stiffness index as the dependent variable, two models with different explanatory power were constructed. In the first model ($R^2 = 0.332$), statistically significant associations were identified between β-stiffness and the following factors: age (β = 0.132; p = 0.018), total cholesterol (β = 5.68; p = 0.003), Framingham cardiovascular risk score (β = 0.40; p = 0.049), and HDL cholesterol (β = −7.10; p < .001). Higher HDL levels were associated with lower arterial stiffness, whereas higher total cholesterol levels and increased cardiovascular risk were associated with increased β-stiffness.

Unexpectedly, triglycerides (β = −2.90; p = 0.007) and LDL cholesterol (β = −5.66; p = 0.004) demonstrated inverse associations with β-stiffness. These findings are contrary to physiological expectations and may reflect the influence of lipid-lowering medications, such as statins, or the presence of unmeasured confounding factors. Treatment with upadacitinib or TNF inhibitors did not show a statistically significant effect compared with the control group.

The second model ($R^2 = 0.129$) exhibited lower predictive value and did not identify statistically significant predictors. The Clinical Disease Activity Index (CDAI) showed a borderline positive association with β-stiffness (β = 0.44; p = 0.072), whereas DAS28-CRP demonstrated an inverse trend (β = −2.88; p = 0.102). Disease duration, treatment duration, steroid dose, inflammatory markers (CRP and ESR), and prior biologic therapy were not significantly associated with arterial stiffness.

Table 56. Multiple regression model for β-stiffness index. Note: R = 0.586; R^2 = 0.332[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	t	p
Intercept[a]	5.445	5.568	0.978	0.330
Treatment group				
TNF inhibitors vs control (1–3)	0.572	1.158	0.494	0.622
Upadacitinib vs control (2–3)	1.826	1.231	1.484	0.141
Age (years)	0.132	0.055	2.398	0.018**
Body mass index (BMI)	0.003	0.103	0.026	0.979
Sex (1 = female, 2 = male)	−3.613	1.879	−1.923	0.057
Current smoking	−0.039	1.058	−0.037	0.971
Total cholesterol	5.680	1.847	3.075	0.003***
Triglycerides	−2.898	1.052	−2.755	0.007***
LDL cholesterol	−5.657	1.923	−2.941	0.004***
HDL cholesterol	−7.098	2.076	−3.419	< .001***
Framingham score (10-year risk of MI or death, %)	0.400	0.200	1.996	0.049*

Table 57. Multiple regression model for the β-stiffness index. Note: R = 0.359; R^2 = 0.129[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	95% CI (Lower)	95% CI (Upper)	*t*	*p*
Intercept[a]	10.371	4.948	0.497	20.245	2.096	0.040*
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	0.292	1.764	−3.229	3.813	0.166	0.869
Disease duration (years)	0.055	0.115	−0.176	0.285	0.473	0.637
Treatment duration (years)	0.149	0.253	−0.355	0.654	0.590	0.557
Corticosteroid dose	0.016	0.280	−0.543	0.574	0.056	0.956
Previous biologic therapy	0.182	0.898	−1.609	1.973	0.203	0.840
C-reactive protein (CRP)	0.002	0.067	−0.131	0.135	0.027	0.978
Erythrocyte sedimentation rate (ESR)	0.057	0.046	−0.035	0.149	1.245	0.218
DAS28-ESR	−0.389	1.589	−3.558	2.781	−0.245	0.807
DAS28-CRP	−2.876	1.737	−6.342	0.589	−1.656	0.102
Clinical Disease Activity Index (CDAI)	0.444	0.243	−0.041	0.928	1.826	0.072

5.4. Analysis of arterial compliance (AC)

In a multiple linear regression analysis with arterial compliance (AC) as the dependent variable, two models were constructed. In the first model, patients treated with upadacitinib exhibited significantly lower AC compared with the control group (β = −0.1846; p = 0.026), indicating increased arterial stiffness. Sex was also identified as a statistically significant factor, with male patients demonstrating higher arterial compliance compared with females (β = 0.2560; p = 0.042). Although not statistically significant, a trend toward a positive association between higher triglyceride levels and arterial compliance was observed (p = 0.081). This finding is

contrary to established pathophysiological mechanisms and suggests the need for further investigation. In the second regression model, which incorporated clinical and inflammatory variables, the Clinical Disease Activity Index (CDAI) was significantly associated with AC, with higher CDAI values correlating with lower arterial compliance (β = −0.0237; p = 0.049), again suggesting increased arterial stiffness in the context of higher disease activity. In this model, treatment with upadacitinib compared with TNF inhibitors did not exert a significant effect on AC (p = 0.491), nor did other variables including CRP, ESR, DAS28 indices, steroid dose, disease duration, or prior biologic therapy.

Table 58. Multiple regression model for arterial compliance (AC, mm/kPa). Note: R = 0.390; R^2 = 0.152[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	*Estimate (B)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Intercept[a]	*0.720*	*0.369*	*−0.012*	*1.452*	*1.953*	*0.054*
Treatment group						
TNF inhibitors vs control (1–3)	*−0.124*	*0.077*	*−0.276*	*0.028*	*−1.620*	*0.108*
Upadacitinib vs control (2–3)	*−0.185*	*0.082*	*−0.346*	*−0.023*	*−2.265*	*0.026**
Sex (1 = female, 2 = male)	*0.256*	*0.124*	*0.009*	*0.503*	*2.057*	*0.042**
Age (years)	*−0.001*	*0.004*	*−0.008*	*0.007*	*−0.149*	*0.882*
Body mass index (BMI)	*−0.008*	*0.007*	*−0.021*	*0.006*	*−1.163*	*0.248*
Total cholesterol	*−0.175*	*0.122*	*−0.418*	*0.068*	*−1.432*	*0.155*
Triglycerides	*0.123*	*0.070*	*−0.016*	*0.261*	*1.761*	*0.081*
LDL cholesterol	*0.177*	*0.127*	*−0.076*	*0.430*	*1.391*	*0.167*
HDL cholesterol	*0.223*	*0.138*	*−0.050*	*0.496*	*1.620*	*0.108*
Framingham score (10-year risk of MI or death, %)	*−0.017*	*0.013*	*−0.043*	*0.009*	*−1.274*	*0.206*
Current smoking	*−0.033*	*0.070*	*−0.172*	*0.106*	*−0.467*	*0.642*

Table 59. Multiple regression model for arterial compliance (AC, mm/kPa). Note: R = 0.371; R^2 = 0.138[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	*Estimate (β)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Intercept[a]	*0.786*	*0.240*	*0.306*	*1.266*	*3.269*	*0.002***
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	*−0.059*	*0.086*	*−0.230*	*0.112*	*−0.692*	*0.491*
Disease duration (years)	*−0.003*	*0.006*	*−0.014*	*0.008*	*−0.494*	*0.623*
Treatment duration (years)	*−0.015*	*0.012*	*−0.039*	*0.010*	*−1.209*	*0.231*
Steroid dose (prednisolone equivalent)	*0.001*	*0.014*	*−0.027*	*0.028*	*0.041*	*0.968*
Previous biologic therapy	*−0.002*	*0.044*	*−0.089*	*0.085*	*−0.043*	*0.966*
C-reactive protein (CRP)	*−0.0001*	*0.003*	*−0.007*	*0.006*	*−0.025*	*0.980*
Erythrocyte sedimentation rate (ESR)	*0.0001*	*0.002*	*−0.004*	*0.005*	*0.028*	*0.978*
DAS28-ESR	*0.014*	*0.077*	*−0.140*	*0.168*	*0.181*	*0.857*
DAS28-CRP	*0.072*	*0.084*	*−0.097*	*0.240*	*0.852*	*0.397*
Clinical Disease Activity Index (CDAI)	*−0.024*	*0.012*	*−0.047*	*−0.0002*	*−2.008*	*0.049**

5.5. Analysis of the elastic modulus (EP)

In a multiple linear regression analysis, patients treated with upadacitinib demonstrated significantly higher EP values compared with healthy controls (β = 28.03; p = 0.040), indicating increased arterial stiffness. Age was also positively associated with EP (β = 1.79; p = 0.004), in line with the well-established physiological relationship between advancing age and reduced vascular elasticity. Other demographic and biochemical variables, including sex, body mass index, smoking status, and lipid profile parameters, did not show a statistically significant effect. A borderline trend suggesting a protective role of HDL cholesterol on vascular elasticity was observed (p = 0.082).

In a second regression model, longer treatment duration was significantly associated with higher EP values (β = 5.82; p = 0.028), suggesting the accumulation of structural vascular changes over time. In addition, higher disease activity as assessed by the Clinical Disease Activity Index (CDAI) was associated with increased arterial stiffness (β = 4.61; p = 0.049). An interesting and unexpected finding was that higher DAS28-CRP values were associated with lower EP (β = −33.32; p = 0.047), which contradicts anticipated pathophysiological

relationships and may reflect methodological limitations or the influence of confounding factors. In this model, treatment type (upadacitinib versus TNF inhibitors) did not exert a statistically significant effect on EP (p = 0.148).

Table 60. Multiple regression model for elastic modulus (EP, kPa). Note: R = 0.589; R² = 0.347[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	*t*	*p*
Intercept[a]	5.984	61.025	0.098	0.922
Treatment group				
TNF inhibitors vs control (1–3)	6.666	12.687	0.525	0.601
Upadacitinib vs control (2–3)	28.034	13.488	2.078	0.040*
Age (years)	1.790	0.604	2.963	0.004**
Body mass index (BMI)	0.870	1.126	0.772	0.442
Current smoking	9.039	11.593	0.780	0.437
Sex (1 = female, 2 = male)	−25.873	20.597	−1.256	0.212
Total cholesterol	24.269	20.246	1.199	0.234
Triglycerides	−16.906	11.529	−1.466	0.146
LDL cholesterol	−18.541	21.077	−0.880	0.381
HDL cholesterol	−40.030	22.754	−1.759	0.082
Framingham score (10-year risk of MI or death, %)	3.398	2.197	1.547	0.125

61. Multiple regression model for elastic modulus (EP, kPa). Note: R = 0.425; R² = 0.180[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	*Estimate (β)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Intercept[a]	***98.746***	***41.250***	***16.463***	***181.028***	***2.394***	***0.019****
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	***25.896***	***17.700***	***−9.418***	***61.209***	***1.463***	***0.148***
Disease duration (years)	***−0.909***	***1.190***	***−3.291***	***1.473***	***−0.761***	***0.449***
Treatment duration (years)	***5.823***	***2.600***	***0.633***	***11.012***	***2.238***	***0.028****
Steroid dose (prednisolone equivalent)	***−0.390***	***2.850***	***−6.078***	***5.297***	***−0.137***	***0.892***
Current smoking	***−1.922***	***15.140***	***−32.126***	***28.282***	***−0.127***	***0.899***
Previous biologic therapy	***5.050***	***9.650***	***−14.211***	***24.311***	***0.523***	***0.603***
DAS28-ESR	***11.243***	***12.180***	***−13.047***	***35.533***	***0.923***	***0.359***
DAS28-CRP	***−33.315***	***16.450***	***−66.122***	***−0.507***	***−2.026***	***0.047****
Clinical Disease Activity Index (CDAI)	***4.608***	***2.290***	***0.031***	***9.186***	***2.008***	***0.049****

5.6. Analysis of asymmetric dimethylarginine (ADMA)

In a multiple linear regression analysis with ADMA as the dependent variable, current smoking was the only statistically significant predictor, being associated with higher ADMA levels—a marker of endothelial dysfunction (β = 0.197; p < .001 in the first model; β = 0.177; p = 0.001 in the second model). This finding is consistent with the well-established relationship between cigarette smoking and endothelial injury.

In both models, no significant differences in ADMA levels were observed between patients treated with upadacitinib or TNF inhibitors and healthy controls. All comparisons regarding treatment type (TNF inhibitors vs controls; upadacitinib vs controls; upadacitinib vs TNF inhibitors) were statistically non-significant (all p > .27). Other variables included in the models—age, body mass index, lipid profile parameters, Framingham cardiovascular risk score, inflammatory markers (CRP and ESR), and disease activity indices (DAS28 and CDAI)—did not show significant associations with ADMA concentrations.

Overall, smoking status emerged as the only consistent and significant predictor of elevated ADMA levels in this analysis. Neither the type of biologic therapy nor inflammatory disease activity exerted a significant influence on this marker of endothelial dysfunction within the studied cohort.

Table 62. Multiple regression model for asymmetric dimethylarginine (ADMA, µmol/L). Note: R = 0.389; R^2 = 0.151[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	*Estimate (B)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Intercept[a]	*0.3739*	*0.2786*	*−0.1791*	*0.9269*	*1.342*	*0.183*
Treatment group						
TNF inhibitors vs controls (1–3)	*−0.0004*	*0.0579*	*−0.1154*	*0.1146*	*−0.007*	*0.995*
Upadacitinib vs controls (2–3)	*−0.0427*	*0.0616*	*−0.1649*	*0.0795*	*−0.693*	*0.490*
Age (years)	*0.0045*	*0.0028*	*−0.0009*	*0.0100*	*1.648*	*0.103*
Sex (1 = female, 2 = male)	*0.1306*	*0.0940*	*−0.0560*	*0.3173*	*1.389*	*0.168*
Body mass index (BMI)	*0.0004*	*0.0051*	*−0.0098*	*0.0106*	*0.082*	*0.935*

Predictor	*Estimate (β)*	*SE*	*95% CI (Lower)*	*95% CI (Upper)*	*t*	*p*
Current smoking	*0.1971*	*0.0529*	*0.0921*	*0.3022*	*3.724*	*< .001**
Framingham 10-year risk of MI or death (%)	*−0.0134*	*0.0100*	*−0.0333*	*0.0065*	*−1.336*	*0.185*
Total cholesterol	*0.0225*	*0.0924*	*−0.1609*	*0.2060*	*0.244*	*0.808*
Triglycerides	*−0.0104*	*0.0526*	*−0.1149*	*0.0941*	*−0.198*	*0.844*
LDL cholesterol	*−0.0311*	*0.0962*	*−0.2221*	*0.1599*	*−0.323*	*0.748*
HDL cholesterol	*−0.0250*	*0.1039*	*−0.2312*	*0.1812*	*−0.241*	*0.810*

Table 63. Multiple linear regression model for asymmetric dimethylarginine (ADMA, µmol/L). Note: R = 0.464; R² = 0.216[a]. [a]Reference category indicated where applicable. p < .05, p < .01, p < .001.

Predictor	Estimate (β)	SE	95% CI (Lower)	95% CI (Upper)	*t*	*p*
Intercept[a]	0.8482	0.1861	0.4768	1.2197	4.558	< .001***
Treatment group						
Upadacitinib vs TNF inhibitors (2–1)	−0.0726	0.0661	−0.2044	0.0593	−1.099	0.276
Disease duration (years)	−0.0015	0.0043	−0.0101	0.0071	−0.347	0.730
Treatment duration (years)	0.0007	0.0095	−0.0182	0.0195	0.070	0.944
Steroid dose (prednisolone equivalent)	0.0037	0.0106	−0.0174	0.0249	0.350	0.727
Current smoking	**0.1773**	0.0532	**0.0710**	**0.2835**	**3.331**	**0.001***
Previous biologic therapy	0.0357	0.0340	−0.0323	0.1036	1.047	0.299
C-reactive protein (CRP)	−0.0014	0.0025	−0.0064	0.0036	−0.546	0.587
Erythrocyte sedimentation rate (ESR)	0.0031	0.0017	−0.0003	0.0065	1.799	0.077
DAS28-ESR	−0.0664	0.0597	−0.1855	0.0527	−1.113	0.270
DAS28-CRP	−0.0159	0.0653	−0.1463	0.1146	−0.243	0.809
Clinical Disease Activity Index (CDAI)	0.0046	0.0091	−0.0135	0.0228	0.510	0.612

V. DISCUSSION

In the present study, a comprehensive evaluation of the effects of biologic and targeted synthetic therapies on vascular parameters in patients with rheumatoid arthritis (RA) was performed. The primary focus was placed on non-invasive ultrasound-based assessment of arterial stiffness, investigation of the serum biomarker of endothelial dysfunction asymmetric dimethylarginine (ADMA), and comparison with clinical disease activity assessed by established indices (DAS28-CRP, DAS28-ESR, and CDAI). The increased cardiovascular risk observed in patients with rheumatoid arthritis represents a major contributor to morbidity and

mortality, and its effective management constitutes a key objective in the comprehensive care of this population. The concept of atherosclerosis as an immune-mediated process has long been discussed. This hypothesis was first proposed by Virchow in the 19th century and was later further developed by Ross through the “response-to-injury” inflammatory hypothesis of atherosclerosis [Raggi P. et al. Atherosclerosis. 2018;276:98–108; Ross R. et al. N Engl J Med. 1976;295:369–377]. This concept has been supported by studies demonstrating a beneficial effect of immune modulation on cardiovascular risk. The LoDoCo2 trial demonstrated the efficacy of low-dose colchicine (0.5 mg daily) in reducing cardiovascular events in a cohort of 5,522 patients, of whom 2,762 were randomized to receive colchicine and 2,760 placebo. Significantly fewer cardiovascular events were observed in the colchicine-treated group compared with the placebo group (2.5 vs. 3.6 events per 100 person-years; hazard ratio [HR] 0.69; 95% confidence interval [CI], 0.57–0.83; $p < 0.001$) [Nidorf SM et al. N Engl J Med. 2020;383:1838–1847]. Similarly, in the CANTOS trial, anti-inflammatory therapy with the interleukin-1 inhibitor canakinumab administered at a dose of 150 mg every three months resulted in a statistically significant reduction in recurrent cardiovascular events in a cohort of 10,061 patients with a prior myocardial infarction and elevated high-sensitivity C-reactive protein (hsCRP) levels (HR vs. placebo, 0.83; 95% CI, 0.73–0.95; $p = 0.005$) [Ridker PM et al. N Engl J Med. 2017;377:1119–1131]. These studies stimulated substantial interest in anti-inflammatory therapies for the treatment of atherosclerosis, and additional mechanisms continue to be explored. For example, investigation of the interleukin-6 inhibitor ziltivekimab has demonstrated efficacy in reducing various surrogate markers of atherosclerosis in a phase II trial, although its clinical effectiveness remains to be established [Wada Y et al. J Cardiol. 2023;82:279–285]. It should be noted, however, that these promising results cannot be readily extrapolated to immunomodulatory therapies used in rheumatoid arthritis. The anti-atherosclerotic treatments discussed above primarily target the innate immune response mediated by the NLRP3 inflammasome [Grebe A et al. Circ Res. 2018;122:1722–1740], whereas RA therapies predominantly target the adaptive immune system. A study investigating etanercept in patients following myocardial infarction did not demonstrate a cardiovascular benefit [Padfield GJ et al. Heart. 2013;99:1330–1335], and another study showed that tocilizumab was not effective in reducing infarct size during the acute phase of myocardial infarction [Broch K et al. J Am Coll Cardiol. 2021;77:1845–1855]. Taken together, the effects of biologic and targeted synthetic therapies on the cardiovascular system remain incompletely understood and represent a clinically relevant and scientifically intriguing area for further investigation.

1. Differences Between the Study Groups

In this study, we sought to evaluate whether significant differences in markers of vascular damage exist between three groups: patients receiving biologic therapy with TNF inhibitors, patients treated with the selective JAK1/2 inhibitor upadacitinib, and a group of healthy controls. The groups were carefully selected and did not differ significantly with respect to sex, age, smoking status, body mass index, or Framingham Risk Score (FRS). The two treatment groups were also statistically comparable in terms of disease activity and disease duration. Despite this overall comparability, several important differences between the study groups were observed. The duration of biologic therapy was significantly longer in the TNF inhibitor group, with a mean treatment duration of 5.36 years, compared with 2.07 years in the upadacitinib group ($p < 0.001$). This difference is largely attributable to the more recent approval and reimbursement of upadacitinib in Bulgaria (October 2020; NHIF decision No. 22650/30.10.2020), whereas TNF inhibitors have been fully reimbursed and widely used in clinical practice since 2012. There is evidence suggesting that prolonged treatment with TNF inhibitors may reduce the risk of cardiovascular events. In a large observational study by Low et al., involving 11,200 patients with RA treated with TNF inhibitors and compared with patients receiving conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), TNF inhibitor–treated patients demonstrated a 39% lower risk of myocardial infarction after a mean treatment duration of 5.3 years (hazard ratio [HR] 0.61; 95% CI 0.41–0.89). No significant differences were observed between the groups in infarct severity or six-month mortality following the event [Low AS et al. Ann Rheum Dis. 2017;76:654–660]. These findings suggest that longer exposure to TNF inhibitors may confer a more favorable vascular effect compared with shorter treatment durations. Another notable difference between the treatment groups was prior exposure to biologic therapies. Patients treated with upadacitinib more frequently had a history of previous biologic treatment compared with those in the TNF inhibitor group. Specifically, 39.2% of patients in the TNF inhibitor group were biologic-naïve, compared with only 22.8% in the upadacitinib group. This difference may reflect established rheumatology practices in Northeastern Bulgaria, including a greater tendency to use JAK inhibitors as second-line therapy. Consequently, patients treated with upadacitinib may represent a population with more difficult-to-treat RA and potentially longer cumulative periods of active disease. Supporting this notion, data from the Canadian Rheumatology Registry indicate reduced drug retention rates for both biologic and targeted synthetic DMARDs in RA patients with cardiovascular risk factors, with insufficient disease control being the primary reason for treatment discontinuation [Aboulenain S et al. ACR Open

Rheumatol. 2023;5:712–717]. Furthermore, recurrent inflammatory flares of RA have been shown to independently increase the risk of cardiovascular events, underscoring the importance of sustained disease control [Myasoedova E et al. Ann Rheum Dis. 2016;75:560–565]. Additional differences were observed in the use of concomitant immunosuppressive therapies. Although no statistically significant difference in corticosteroid use was identified (mean dose 1.37 mg in the TNF inhibitor group vs. 1.65 mg in the upadacitinib group; p = 0.379), significant differences were noted in the use of concomitant csDMARDs. In the TNF inhibitor group, more than half of the patients were receiving methotrexate (53%, n = 22), compared with only 13.2% in the upadacitinib group. Conversely, the majority of patients treated with upadacitinib were receiving monotherapy (78.9%), compared with 41.5% in the TNF inhibitor group. These differences are partly expected, given current recommendations favoring combination therapy with methotrexate and TNF inhibitors, as well as evidence supporting comparable efficacy of upadacitinib monotherapy versus combination therapy [Sanmartí R, Corominas H. J Clin Med. 2023;12:1734]. Nevertheless, methotrexate itself may exert independent effects on arterial stiffness, vascular dysfunction, and overall cardiovascular risk. Several studies have demonstrated that methotrexate monotherapy can improve endothelial dysfunction [Cafaro G et al. Arthritis Res Ther. 2022;24:236]. In experimental and clinical settings, methotrexate has also been shown to promote vascular healing after stent implantation and to exert cardioprotective effects across various inflammatory rheumatic diseases [Verhoeven F et al. Expert Rev Clin Pharmacol. 2021;14:1105–1112; Liu X et al. Cardiovasc Drugs Ther. 2021;35:915–925]. Conversely, there is evidence suggesting that methotrexate may accelerate atherosclerosis through inhibition of folate metabolism, highlighting the complex and potentially bidirectional vascular effects of this therapy [Onishi Y et al. In Vivo. 2025;39:1262–1274].

2. Effect of biological agents on lipid metabolism

The comparative analysis demonstrated higher levels of total cholesterol, LDL, and HDL in the group treated with upadacitinib compared with both the control group and the TNF inhibitor group. The increase in lipoproteins is a class effect of JAK inhibitors and is mainly related to modulation of IL-6 signaling and its impact on hepatocyte function [Li N et al., Clin Rheumatol. 2022;41:689–693]. This cholesterol increase occurs concurrently in both low- and high-density lipoproteins and does not alter the LDL/HDL ratio. In patients with inflammatory diseases, disease activity leads to paradoxical changes in lipoproteins, described in the literature as the “lipid paradox.” In rheumatoid arthritis (RA), patients often exhibit low levels

of total cholesterol (both LDL and HDL) despite an increased cardiovascular risk—a phenomenon known as the lipid paradox [Venetsanopoulou AI et al., Rheumatol Int. 2020;40:1181–1191]. This is primarily attributed to chronic inflammation, which accelerates cholesterol catabolism via pro-inflammatory cytokines such as TNF-α and IL-6. These cytokines increase the expression of hepatic receptors (LDLR and SR-B1), enhancing LDL uptake and degradation. Treatment with JAK inhibitors and IL-6 blockers (e.g., tocilizumab) reduces inflammation, decreases cholesterol catabolism, and leads to increased lipid levels. The effect of TNF inhibitors on lipid metabolism appears to differ from that of JAK inhibitors. A literature review and meta-analysis including 32 studies, 13 of which were prospective, showed that TNF inhibitors increase HDL levels without affecting LDL [Daïen CI et al., Ann Rheum Dis. 2012;71:862–868]. Interestingly, lipid profile changes in RA correlate more strongly with CRP levels than with clinical disease activity indices (e.g., DAS28), underscoring the central role of inflammation in this metabolic paradox. Our regression analyses also revealed notable effects of lipid parameters on markers of vascular damage. Higher HDL levels were associated with lower PWV ($\beta = -1.81$; $p = 0.005$) and lower β-stiffness index ($\beta = -7.10$; $p < 0.001$), while a trend toward an inverse association with EP was observed ($p = 0.082$). Low HDL levels have been linked to increased arterial stiffness in the general population [Wang X et al., PLoS One. 2013;8:e81778]. The inverse relationship between RA disease activity and HDL levels has also been described in the literature [Choudhury C et al., Cureus. 2024]. RA and chronic inflammation profoundly alter HDL metabolism, contributing to increased cardiovascular risk. In patients with active RA, reduced HDL-C levels are observed, leading to a higher atherogenic index (TC/HDL-C). Beyond quantitative changes, qualitative alterations also occur—HDL loses its protective functions, including cholesterol efflux from macrophages, as well as its antioxidant and anti-inflammatory properties [Su X et al., Mol Biol Rep. 2021;48(7):5723–5733]. Under chronic inflammatory conditions, HDL may transform into a dysfunctional particle with pro-atherogenic properties. Pro-inflammatory cytokines such as TNF-α and IL-6 play a central role in this process by disrupting the expression and function of key enzymes and receptors involved in HDL metabolism and reverse cholesterol transport. Collectively, these changes promote the development of atherosclerosis in RA. Therefore, it may be theorized that the elevated cholesterol levels observed during treatment with upadacitinib reflect effective suppression of inflammatory activity and may paradoxically indicate a reduction in cardiovascular risk.

3. Effect of Biologic and Targeted Synthetic Therapy on Markers of Arterial Stiffness

In the present study, we compared the degree of arterial stiffness among three groups: patients treated with TNF inhibitors, patients treated with upadacitinib, and a group of healthy controls. Arterial stiffness parameters assessed by the echo-tracking method did not differ significantly between the TNF inhibitor group and healthy controls. These findings suggest that the immunomodulatory effects of TNF inhibitors may improve arterial stiffness in patients with rheumatoid arthritis, resulting in values comparable to those observed in healthy individuals. In this cross-sectional analysis, no statistically significant differences were identified between the TNF inhibitor group and controls across the evaluated parameters: pulse wave velocity (PWV) 6.417 m/s vs. 5.878 m/s ($p = 0.378$), augmentation index (AI%) 19.390 vs. 18.33 ($p = 0.927$), β-stiffness 8.720 vs. 7.280 ($p = 0.510$), arterial compliance (AC) 0.708 vs. 0.861 ($p = 0.110$), and elastic modulus (EP) 113.024 vs. 94.367 ($p = 0.340$). These results contrast with data from multiple meta-analyses demonstrating significantly increased arterial stiffness in patients with rheumatoid arthritis compared with healthy controls. A meta-analysis including 25 studies with a total of 1,472 RA patients and 1,583 controls reported a mean difference in PWV of 1.32 m/s (95% CI 0.77–1.88; $p < 0.00001$) [Ambrosino P et al., Ann Med. 2015;47:457–467]. In the same analysis, six studies involving 214 RA patients and 327 controls demonstrated a significantly higher augmentation index (AIx) in RA patients compared with controls (weighted mean difference [WMD] 11.50%; 95% CI 5.15–17.86; $p = 0.0004$). Importantly, a subgroup analysis of patients with early RA also showed significantly increased aortic PWV compared with controls. A more recent meta-analysis focusing primarily on PWV and including 38 studies (2,733 RA patients and 2,416 healthy controls) reported similar findings [Wang P et al., Arch Med Res. 2019;50:401–412]. Patients with RA exhibited significantly higher carotid–femoral PWV (WMD 1.10 m/s; 95% CI 0.84–1.35), brachial–ankle PWV (WMD 0.20 m/s; 95% CI 0.12–0.28), and carotid–radial PWV (WMD 0.51 m/s; 95% CI 0.23–0.79). Augmentation index values were also significantly elevated in RA patients, both for AIx (WMD 4.79%; 95% CI 1.34–8.24) and for heart rate–corrected AIx at 75 beats per minute (AIx@75; WMD 5.78%; 95% CI 3.82–7.74). Subgroup regression analysis in this meta-analysis identified significant associations between carotid–femoral PWV and age, disease duration, and erythrocyte sedimentation rate (ESR) in RA patients. In contrast to data derived from cohorts with active rheumatoid arthritis, our results indicate that although markers of aortic stiffness were numerically higher in TNF inhibitor–treated patients, they did not differ significantly from those observed in healthy controls. These findings are consistent with studies investigating the effects of TNF inhibitors on arterial stiffness. In our previous literature review, 10 of 13 published studies demonstrated a reduction in arterial stiffness following initiation of

TNF inhibitor therapy [Gerganov G et al., Clin Rheumatol. 2023]. Furthermore, a meta-analysis by Abdulmajid et al., encompassing 23 studies, reported a mean reduction in PWV of 0.51 m/s and a reduction in AIx of −0.57% following TNF inhibitor therapy [Abdulmajid B et al., Clin Rheumatol. 2023;42:999–1011]. In contrast, a systematic review by Knowles et al., including 27 studies (22 assessing PWV and 19 assessing AIx), found that only a minority demonstrated significant improvement after anti–TNF-α therapy, while randomized controlled trials failed to show a clear benefit [Knowles L et al., Br J Clin Pharmacol. 2020;86:837–851]. Reported reductions in PWV in studies with positive findings ranged from 0.46 to 2.6 m/s, whereas effects on AIx were limited and inconsistent. Overall, these findings suggest that effective long-term TNF inhibitor therapy may attenuate arterial stiffness in patients with rheumatoid arthritis, potentially normalizing vascular parameters to levels comparable with those of healthy individuals.

4. Arterial Stiffness in Patients Treated with Upadacitinib

The comparative analysis of arterial stiffness markers between the upadacitinib-treated group and healthy controls demonstrated a tendency toward worsening vascular parameters in the treated group, with two markers reaching statistical significance. Specifically, pulse wave velocity (PWV) was higher in the upadacitinib group compared with controls (6.811 vs. 5.878 m/s; $p = 0.063$), β-stiffness was also higher (9.511 vs. 7.280; $p = 0.213$), and the augmentation index (AI%) showed a nonsignificant increase (25.574 vs. 18.33; $p = 0.112$). In contrast, arterial compliance (AC) was significantly lower in the upadacitinib group (0.652 vs. 0.861; $p = 0.020$), and the elastic modulus (EP) was significantly higher (130.421 vs. 94.367; $p = 0.017$), both indicating increased arterial stiffness. Our regression models further supported an association between upadacitinib therapy and markers of increased arterial rigidity. In the model using PWV as the dependent variable, treatment with upadacitinib emerged as a significant predictor of higher PWV values compared with the control group ($\beta = 0.80$; $p = 0.035$). Analysis with AI% as the dependent variable did not reveal statistically significant differences between therapeutic groups, although a trend toward higher AI% values was observed in patients receiving upadacitinib ($\beta = 6.28$; $p = 0.118$). In the β-stiffness model, no significant effect of upadacitinib treatment was identified relative to controls. With respect to arterial compliance, upadacitinib therapy was associated with significantly lower AC compared with healthy controls ($\beta = -0.1846$; $p = 0.026$), again suggesting increased arterial stiffness. Finally, in the model using elastic modulus (EP) as the outcome variable, patients treated with upadacitinib exhibited significantly higher EP values than controls ($\beta = 28.03$; $p = 0.040$). Taken together,

these findings suggest impaired vascular function in patients treated with the JAK inhibitor upadacitinib. Although not unequivocal, our results indicate that different immunosuppressive agents are not interchangeable with respect to their cardiovascular effects. Data addressing this issue in the available literature are scarce. We identified only one study that examined PWV in patients treated with JAK inhibitors after three months of therapy [Anyfanti P et al., Diagnostics. 2024;14:834]. In that study, no significant effects were observed on blood pressure, arterial stiffness, or carotid intima–media thickness (cIMT), with the exception of increased nocturnal PWV. Notably, however, the authors reported alterations in microvascular circulation assessed by capillaroscopy, a finding that warrants further investigation. From a pathophysiological perspective, JAK–STAT signaling is thought to play a central role in the development of multiple cardiovascular diseases [Kishore R, Verma SK. JAKSTAT. 2012;1:118–124]. In particular, STAT1 and STAT3 are involved in the regulation of inflammatory responses, endothelial dysfunction, vascular wall remodeling, and cardiac hypertrophy. Activation of STAT1 is generally associated with pro-apoptotic and pro-inflammatory effects, whereas STAT3 exerts a more protective role in cardiac tissue. STAT3 is considered a key mediator of cardiovascular protection by suppressing inflammation, limiting cell death, and promoting angiogenesis. In animal models with deletion of the STAT3-encoding gene, increased cardiac apoptosis and fibrosis have been observed, along with elevated secretion of inflammatory cytokines such as TNF-α [Yu Z et al., Biochem J. 2002;367:97–105; Bolli R et al., J Mol Cell Cardiol. 2011;50:589–597]. Moreover, STAT3 activates vascular endothelial growth factor (VEGF) expression in cardiomyocytes, thereby supporting neovascularization after myocardial infarction. This effect is further enhanced by IL-10, which improves microcirculation and survival of endothelial progenitor cells via STAT3 signaling [Krishnamurthy P et al., Circ Res. 2011;109:1280–1289]. Overall, STAT3 plays a pivotal cardioprotective role through modulation of inflammation, vascular regeneration, and cellular resilience. Dysregulation of STAT signaling may therefore lead to chronic endothelial dysfunction, increased arterial stiffness, and the development of atherosclerosis. The relative selectivity of upadacitinib for JAK1 compared with JAK2/3 and TYK2 adds further complexity to the pathophysiological landscape. Importantly, this selectivity is not absolute, and some degree of inhibition of other tyrosine kinases has been demonstrated [Traves PG et al., Ann Rheum Dis. 2021;80:865–875]. The treatment-associated changes observed in arterial stiffness markers—namely increased PWV and EP and reduced AC—may reflect deeper mechanisms involving JAK–STAT signaling, including potential dysregulation of protective STAT3-mediated pathways and amplification of STAT1-associated inflammatory responses. These

hypotheses underscore the need for future studies to clarify the long-term cardiovascular profile of JAK inhibitors in the context of chronic inflammatory diseases such as rheumatoid arthritis.

5. Comparison of Arterial Stiffness Results Between the Two Treatment Groups

When comparing arterial stiffness parameters between the TNF inhibitor (TNFi) group and the upadacitinib group, no statistically significant differences were observed between the two treatments: PWV 6.417 vs. 6.811 m/s ($p = 0.539$); beta stiffness 8.720 vs. 9.511 ($p = 0.793$); AI% 19.390 vs. 25.574 ($p = 0.241$); arterial compliance (AC) 0.708 vs. 0.652 ($p = 0.710$); and elastic modulus (EP) 113.024 vs. 130.421 ($p = 0.529$). The regression analyses likewise did not reveal significant differences for most arterial stiffness markers. In the PWV model, the difference between groups did not reach statistical significance ($\beta = 0.319$; $p = 0.555$). With respect to AI%, patients treated with upadacitinib showed a trend toward higher values compared with those receiving TNF inhibitors ($\beta = 7.75$; $p = 0.119$), although this difference was not statistically significant. No meaningful difference was observed for the beta stiffness index between the two therapeutic groups ($\beta = 0.292$; $p = 0.869$). Similarly, for arterial compliance, upadacitinib treatment was not associated with lower vascular elasticity compared with TNF inhibitors ($\beta = -0.059$; $p = 0.491$). Only for EP was a borderline trend toward higher values observed in the upadacitinib group compared with TNF inhibitors ($\beta = 25.89$; $p = 0.148$), but this result did not reach statistical significance. Whether there is a difference in early vascular changes between JAK inhibitors and TNF inhibitors remains an insufficiently studied question, and to our knowledge, there are no published data directly addressing this issue. Concerns that JAK inhibitors may be associated with increased cardiovascular risk originate primarily from the ORAL Surveillance trial [Charles-Schoeman C, Buch MH, Dougados M, et al. Ann Rheum Dis. 2023;82:119–129]. This study evaluated the long-term safety of tofacitinib compared with TNF inhibitors in patients with rheumatoid arthritis older than 50 years who had at least one additional cardiovascular risk factor. The results showed that in patients with a history of atherosclerotic cardiovascular disease, treatment with tofacitinib (5 or 10 mg twice daily) was associated with a significantly higher risk of major adverse cardiovascular events (MACE), including myocardial infarction and sudden cardiac death, compared with TNF inhibitors. In contrast, among patients without prior cardiovascular events but with existing risk factors, no significant difference in risk between the two therapies was observed, although a small increase in absolute risk with JAK inhibitors could not be completely excluded. An important question is whether these findings can be extrapolated as a class effect of all JAK inhibitors or whether they are directly related to the relatively non-selective inhibition of

JAK1–3 and TYK2 by tofacitinib. The cardiovascular impact of different selective JAK inhibitors remains unclear due to the lack of head-to-head comparative trials; however, several population-based studies provide relevant insights. Data from the Swedish ARTIS program did not identify an increased risk of major cardiovascular events in patients with RA treated with tofacitinib or baricitinib, with baricitinib even being associated with a lower risk of acute coronary syndrome compared with patients receiving the TNF inhibitor etanercept [Frisell T, Bower H, Morin M, et al. Ann Rheum Dis. 2023;82:601–610]. Similarly, Hoisnard and colleagues reported low incidences of MACE in patients treated with tofacitinib (2.8 per 1,000 patient-years) or baricitinib (5.2 per 1,000 patient-years) [Hoisnard L, Pina Vegas L, Dray-Spira R, et al. Ann Rheum Dis. 2023;82:182–188]. A systematic review and meta-analysis by Xie et al. demonstrated that tofacitinib, baricitinib, and upadacitinib were not associated with a significant increase in MACE risk compared with placebo across 26 randomized controlled trials, although many of these studies were of relatively short duration [Xie W, Huang Y, Xiao S, et al. Ann Rheum Dis. 2019;78:1048–1054]. A more recent systematic review and meta-analysis by Wei and colleagues likewise found no statistically significant difference in MACE incidence among different JAK inhibitors [Wei Q, Wang H, Zhao J, et al. Front Pharmacol. 2023;14]. Another comparative analysis examined the safety of upadacitinib versus TNF inhibitors by stratifying patients into low- and high-risk MACE groups. In a post hoc analysis of the SELECT-COMPARE trial, the benefit–risk balance of upadacitinib (15 mg) versus adalimumab (40 mg) was assessed in patients with rheumatoid arthritis and differing cardiovascular risk profiles: patients younger than 65 years without cardiovascular risk factors and patients older than 65 years with one or more cardiovascular risk factors [Fleischmann R, Curtis JR, Charles-Schoeman C, et al. Ann Rheum Dis. 2023;82(9):1130–1141]. The results showed that in both low- and high-risk patients, the incidence of serious events—including malignancies, MACE, and venous thromboembolism—was comparable between the two therapies, although slightly higher in the group with elevated cardiovascular risk. In summary, although patients treated with upadacitinib consistently demonstrated numerically less favorable arterial stiffness parameters compared with those treated with TNF inhibitors, these differences did not reach statistical significance. This pattern suggests a potential trend toward a more adverse vascular effect of JAK inhibition, which warrants further investigation in larger, adequately powered studies.

6. ADMA Impact on the Endothelial Dysfunction Marker ADMA

Our comparative analysis did not demonstrate significant differences in ADMA levels among the three study groups ($F = 0.432$, $df = 2$, $p = 0.651$). Correlation analysis did not identify significant associations with disease duration, the type of medication used, or disease activity; however, a significant relationship with current smoking was observed (Spearman's $\rho = 0.332$, $p = 0.034$). This association was also confirmed in both regression models ($\beta = 0.197$; $p < .001$ in the first model; $\beta = 0.177$; $p = 0.001$ in the second model). A similar association has been reported by other authors, with smoking leading to substantially higher ADMA levels—up to 80% higher compared with non-smokers [Zhang WZ, Venardos K, Chin-Dusting J, Kaye DM. Hypertension. 2006;48(2):278–285]. This effect appears to be directly related to nicotine-mediated modulation of the DDAH/ADMA/NOS pathway and has been linked not only to increased cardiovascular risk but also to other smoking-related adverse outcomes, such as erectile dysfunction and cerebral microvascular injury [Jiang DJ et al. Biochem Biophys Res Commun. 2006;349:683–693; Tostes RC et al. J Sex Med. 2008;5:1284–1295; Gao Q et al. J Neurol Sci. 2015;354:27–32]. The relationship between ADMA and rheumatoid arthritis has also been investigated in considerable detail. In a meta-analysis including 16 studies and 1,365 participants, ADMA levels were significantly elevated in RA patients (standardized mean difference [SMD] = 0.84; 95% CI 0.32–1.35) [Zhao CN et al. Amino Acids. 2019;51:773–782]. The increase was more pronounced in patients with body mass index (BMI) ≥ 24, disease duration ≥ 8 years, age < 50 years, and moderate disease activity (DAS28 between 3.2 and 5.1). Interestingly, among older patients (≥ 50 years), those with shorter disease duration (< 8 years), or those with high disease activity (DAS28 ≥ 5.1), no substantial difference was detected. It should be noted that this meta-analysis reported substantial heterogeneity ($I^2 = 93.70\%$, $p < 0.001$), which complicates interpretation. Another meta-analysis of 14 studies including 1,473 participants similarly reported significantly higher serum/plasma ADMA levels in RA patients compared with healthy controls (SMD = 1.02; 95% CI 0.49–1.55). In that analysis, ADMA levels were associated with age, with elevations observed only in patients older than 50 years compared with controls (SMD = 1.48; 95% CI 0.67–2.02). No associations with disease duration or disease activity were observed ($\beta = 0.019$, $p = 0.782$; and $\beta = 0.161$, $p = 0.757$, respectively) [Zafari P et al. Clin Rheumatol. 2020;39(1):127–134]. An important question concerns the effect of RA treatment on this marker of endothelial dysfunction. There is evidence that low-dose prednisolone is associated with reduced ADMA levels in RA patients [Radhakutty A et al. Atherosclerosis. 2017;266:190–195]. Although there is a pathophysiological rationale for methotrexate to reduce ADMA concentrations—via increased consumption of 5-methyltetrahydrofolate, followed by inhibition of homocysteine

remethylation to methionine—such an effect has not yet been demonstrated in observational studies of methotrexate therapy [Turiel M et al. Cardiovasc Ther. 2010; Fiskerstrand T et al. J Pharmacol Exp Ther. 1997]. Several studies assessing the impact of TNF inhibitors on ADMA have reported reductions in RA patients after treatment initiation, whereas one study found no effect on ADMA following initiation of tofacitinib therapy.

7. Effect of Age and Sex on Markers of Vascular Damage

Age correlated with markers of vascular damage across all three study groups and emerged as a major predictor of changes in arterial stiffness in the regression analyses. In our cohort, male sex was correlated with arterial compliance (AC) in patients treated with TNF inhibitors (ρ = 0.466, p = 0.002), with AI% in the control group ($\rho = -0.364$, p = 0.048), while no significant sex-related correlations were identified in the upadacitinib group. Male sex also showed a positive association with AC in the regression analysis (β = 0.2560; p = 0.042). For the other vascular damage markers, only non-significant trends were observed. These heterogeneous findings regarding sex are most likely related to the very small number of male participants included in our study. The associations between arterial stiffness, age, and sex are well documented in the literature. Arterial stiffness is strongly influenced by both age and sex. Data from large population-based studies demonstrate that pulse wave velocity (PWV) increases progressively with advancing age in both men and women [Lu Y et al. EBioMedicine. 2023;92:104619]. In younger age groups (20–40 years), men exhibit higher values of both brachial–ankle PWV (baPWV) and carotid–femoral PWV (cfPWV), reflecting a more rapid loss of arterial wall elasticity compared with women. However, this difference diminishes with advancing age. For baPWV, convergence between the sexes begins around the age of 60 and becomes particularly pronounced after 70 years, when women experience a sharp acceleration in arterial stiffening. For cfPWV, the sex difference persists more consistently over time but also decreases significantly in older age. This phenomenon is largely explained by hormonal factors: before menopause, women benefit from a degree of vascular protection attributable to estrogen. Estrogen stimulates the expression of endothelial nitric oxide synthase (eNOS), leading to increased nitric oxide (NO) bioavailability, which promotes vasodilation and inhibits proliferation of vascular smooth muscle cells [Chambliss KL & Shaul PW. Endocr Rev. 2002;23(5):665–686]. NO plays a crucial role in vasodilation and in suppressing vascular smooth muscle cell proliferation. With declining estrogen levels during menopause, NO production decreases, contributing to increased vascular tone and arterial stiffness [Iqbal J & Zaidi M. Endocrinology. 2009;150(8):3443–3445]. In addition, menopause is associated with

increased oxidative stress, which accelerates NO degradation and further promotes endothelial dysfunction. Studies comparing postmenopausal women with women who retain regular menstruation demonstrate significantly higher arterial stiffness after adjustment for conventional vascular risk factors, including body mass index, prior cardiovascular events, glomerular filtration rate, diabetes, blood pressure, smoking status, and others [Vallée A. Maturitas. 2025;198:108608]. The age-related worsening of arterial stiffness is particularly relevant to the management of patients with rheumatoid arthritis (RA), given the high cardiovascular mortality in this population. Evidence suggests that vascular age in RA does not correspond to chronological age. Coronary CT angiography studies have shown significantly greater accumulation of calcified plaques in patients with RA compared with age-matched controls, with persistent inflammatory activity identified as a key driver of this process of premature vascular aging [Hansen PR et al. Eur J Intern Med. 2019;62:72–79].

8. Effect of Disease Activity, Disease Duration, and Treatment Duration on Markers of Vascular Damage

In our study, we obtained heterogeneous results regarding the effects of disease activity, disease duration, and treatment duration on markers of vascular damage. Overall, our findings indicate that both disease activity and the chronicity of rheumatoid arthritis (RA) are important contributors to the development of vascular damage in affected patients. In the group treated with TNF inhibitors, disease duration showed significant positive correlations with pulse wave velocity (PWV; $r = 0.369$, $p = 0.018$), β-stiffness ($r = 0.327$, $p = 0.037$), and elastic modulus (EP; $r = 0.368$, $p = 0.018$), as well as a significant negative correlation with arterial compliance (AC; $r = -0.429$, $p = 0.005$). The Clinical Disease Activity Index (CDAI) emerged as an independent predictor of vascular function. Higher CDAI values were associated with a significant increase in PWV ($\beta = 0.155$; $p = 0.039$) and a reduction in AC ($\beta = -0.0237$; $p = 0.049$), indicating a link between active disease and increased arterial stiffness. In addition, treatment duration proved to be a significant factor, with longer therapeutic exposure associated with higher EP values ($\beta = 5.82$; $p = 0.028$). Similarly, higher CDAI scores were also associated with increased EP ($\beta = 4.61$; $p = 0.049$), further supporting the role of active inflammation in vascular wall remodeling. These findings underscore that high disease activity and the cumulative effects of disease and treatment duration are key drivers of progressive deterioration in arterial elasticity and structural vascular damage. In contrast, the composite indices DAS28-CRP and DAS28-ESR did not show significant correlations or independent associations with markers of vascular damage. Likewise, acute-phase reactants (ESR and CRP) were not

significantly correlated with vascular parameters. Disease duration has been identified as a risk factor for early atherosclerosis by other investigators as well. A study by Vázquez-Del Mercado et al., including 106 patients with RA without traditional cardiovascular risk factors, examined the effect of disease duration on arterial stiffness measured by carotid–femoral PWV (cfPWV) [Medicine. 2017;96(33):e7862]. cfPWV correlated positively not only with age ($r = 0.450$; $p < 0.001$) but also with disease duration ($r = 0.340$; $p < 0.001$). Arterial stiffness was significantly higher in patients with disease duration ≥10 years (8.4 ± 1.8 m/s) compared with those with <2 years (7.0 ± 0.8 m/s) or 2–10 years (7.8 ± 1.3 m/s). Multivariable analysis showed that each additional year of RA increased cfPWV by $\beta = 0.072$, compared with $\beta = 0.054$ per year of biological aging. Similarly, a cross-sectional study by Sliem and Nasr involving 63 RA patients found that 75% of those with increased aortic stiffness had disease duration exceeding 10 years [J Cardiovasc Dis Res. 2010;1(3):110–115]. However, other authors have not observed an association between longer disease duration and increased aortic stiffness [Taverner D et al., Sci Rep. 2019;9:4543]. A systematic review and meta-analysis of 38 studies demonstrated that cfPWV is significantly higher in RA patients compared with healthy controls (WMD = 1.10 m/s; 95% CI: 0.84–1.35), with age, disease duration, and ESR levels associated with increased cfPWV [Wang P et al., Arch Med Res. 2019;50:401–412]. Subgroup analyses revealed that patients younger than 50 years, with disease duration <6 years and ESR ≥20 mm/h, had higher cfPWV values compared with older patients (≥50 years), those with disease duration ≥6 years, and ESR <20 mm/h. These findings highlight the complex interplay between inflammatory activity, aging, and cumulative disease burden in accelerating vascular aging in RA, and suggest that disease duration alone is not a reliable predictor of vascular damage at the individual patient level. The impact of disease activity on arterial stiffness in RA remains an important issue. The prospective JointHeart study followed 214 RA patients over three years, assessing arterial stiffness by cfPWV [Linde A et al., Blood Press. 2024;33(1)]. Higher disease activity measured by DAS28-CRP was independently associated with increased cfPWV. Patients with moderate or high DAS28-CRP had significantly higher cfPWV compared with those in remission or with low disease activity. In a multivariable model adjusted for age, sex, BMI, and diabetes, a one-unit increase in DAS28-CRP was associated with an approximately 0.3 m/s increase in cfPWV, supporting the hypothesis that active inflammation accelerates arterial stiffening and increases cardiovascular risk in RA. The meta-analysis by Ambrosino et al., including 25 studies with 1,472 RA patients and 1,583 controls, demonstrated significantly higher values of all arterial stiffness parameters (aortic-PWV, ba-PWV, AIx, and AIx@75) in RA patients compared with controls [Ann Med. 2015;47:457–467]. Meta-regression analysis

showed that DAS28 significantly affected aortic-PWV and AIx, CRP levels influenced AIx and AIx@75, and ESR influenced aortic-PWV. Nevertheless, not all studies confirm a relationship between disease activity and arterial stiffness. In a cross-sectional study of 95 patients, Youssef et al. found only a weak correlation between disease activity and the aortic index [Egypt Heart J. 2018;70:35–40]. An important question is whether achieving remission improves arterial stiffness. In a randomized controlled trial by Tam et al., 120 patients with early RA (<12 months disease duration, DMARD-naïve) were randomized to a treat-to-target strategy aiming for remission defined either by SDAI ≤3.3 or DAS28 <2.6. After 12 months, no significant differences were observed between groups in PWV or AIx. However, post-hoc analysis showed that achieving sustained remission—regardless of the index used—was the strongest predictor of stabilization of vascular function, with significantly lower PWV progression compared with patients who remained in moderate or high disease activity (mean difference −0.22 m/s; p = 0.03). In our study, we observed associations between CDAI and markers of arterial stiffness, but not with DAS28-ESR or CRP. This finding may reflect the specific effects of targeted disease-modifying therapies on composite disease activity indices. The strong suppression of acute-phase reactants by biological and targeted synthetic therapies may distort indices incorporating ESR or CRP. This interpretation is supported by prior research. A meta-analysis by Janke et al. compared DAS28, SDAI, CDAI, and ACR/EULAR Boolean remission across 60 randomized clinical trials involving over 22,000 patients treated with biologics or JAK inhibitors [BMC Rheumatol. 2022;6:82]. Indices including acute-phase reactants (DAS28, and to a lesser extent SDAI) consistently showed larger apparent treatment effects compared with purely clinical indices such as CDAI. This effect was most pronounced with IL-6 inhibitors and also evident, though to a lesser extent, with JAK inhibitors. These findings indicate a mechanistically driven bias: therapies that directly suppress CRP may appear more effective in indices incorporating laboratory parameters, even when clinical improvement is not proportionate. In this context, CDAI—based solely on clinical variables—emerges as a more reliable and less biased tool for comparative effectiveness analyses. Similar conclusions have been drawn by other authors. Santos et al. showed that DAS28-CRP systematically overestimates remission and allows residual disease activity, whereas SDAI and CDAI provide a better balance of sensitivity and specificity relative to Boolean remission [PLoS One. 2022;17:e0273789]. Brkić et al. demonstrated that in the same cohort, only 23% of patients met Boolean remission criteria compared with 73% by DAS28(3)-CRP, with patient global assessment contributing substantially to these discrepancies [Rheumatol Ther. 2022;9:1531–1547]. Collectively, these data emphasize that different composite indices are not

interchangeable and can substantially alter clinical interpretation of treatment efficacy. Indices incorporating acute-phase reactants (DAS28/SDAI) tend to favor therapies that directly suppress CRP or ESR (e.g., IL-6 inhibitors and JAK inhibitors), whereas CDAI provides more conservative and likely less biased estimates. Accordingly, for comparative effectiveness and head-to-head studies in RA, CDAI appears to be the most appropriate primary metric.

9. Strengths and Limitations of the Study

This study has several notable strengths. First, it is innovative, as it is among the first to compare early vascular changes in patients with rheumatoid arthritis treated with TNF inhibitors and upadacitinib. An additional strength is the inclusion of a healthy control group and the comparability of the three studied groups in terms of age, disease activity, and disease duration, which reduces the risk of systematic bias. The focus on aortic stiffness as an early indicator of subclinical atherosclerosis adds practical relevance to the findings and provides a basis for more accurate cardiovascular risk stratification.

At the same time, several important limitations should be acknowledged. Although the single-time-point measurement of pulse wave velocity (PWV) using the Aloka ultrasound system is easier to apply and requires less time and equipment compared with standard techniques, it is not as well validated or widely established in clinical practice as carotid–femoral PWV (cfPWV), which is considered the gold standard. The cross-sectional design of the study does not allow for the establishment of causal relationships and permits only the identification of descriptive associations; therefore, longitudinal observational studies with follow-up over time are needed to validate the present findings. The inclusion of additional serological markers of vascular injury (e.g., vWF, VCAM-1, ICAM-1) would further enhance and broaden the interpretation of the results. The relatively small sample size and the potential influence of uncontrolled confounding factors (such as smoking, metabolic disorders, and concomitant medications) should also be taken into account. For these reasons, future longitudinal observational studies are required to validate and expand upon the current results.

VI. CONCLUSIONS

1. Ultrasound-based indices of arterial stiffness can be used as reliable markers of early vascular damage in patients with rheumatoid arthritis (RA). Higher values were observed in patients treated with upadacitinib compared with healthy controls, and a trend toward increased values was noted in the TNF inhibitor (TNFi) group, although without statistical significance. No statistically significant differences in arterial stiffness were found when the two therapeutic groups (TNFi vs upadacitinib) were directly compared.
2. ADMA levels did not differ between healthy controls and the two treatment groups, nor between TNFi and upadacitinib. ADMA was dependent solely on current smoking status.
3. Treatment with upadacitinib was associated with a characteristic increase in total cholesterol, LDL, and HDL compared with TNFi and controls, reflecting the well-described effects of JAK inhibitors and the lipid paradox in RA. HDL levels demonstrated a protective association with arterial stiffness, whereas the other lipid parameters showed no direct relationship.
4. A relationship was established between arterial stiffness and disease activity, particularly as assessed by the Clinical Disease Activity Index (CDAI), whereas ADMA did not correlate with disease activity. CDAI emerged as the most reliable composite index for reflecting vascular changes.
5. The Framingham Risk Score correlated with indices of arterial stiffness and reflected early vascular damage. Multivariate regression analysis identified disease activity, disease duration, and therapeutic regimen as independent factors associated with vascular damage.

VII. CONTRIBUTIONS

Methodological Contributions

1. This is the first clinical study to directly compare the effects of TNF inhibitors and the JAK inhibitor upadacitinib on early markers of vascular damage in patients with rheumatoid arthritis.

2. An ultrasound-based assessment of arterial stiffness (PWV measured with Aloka) was introduced as an accessible and clinically applicable method for detecting early vascular dysfunction.

Scientific Contributions

1. The reliability of ultrasound-derived arterial stiffness indices as markers of early vascular damage in rheumatoid arthritis was demonstrated.

2. It was established that patients treated with upadacitinib exhibit higher arterial stiffness compared with healthy controls, whereas TNF inhibitors show only a non-significant trend toward a similar effect.

3. It was shown that upadacitinib leads to a characteristic increase in total cholesterol, LDL, and HDL.

4. It was demonstrated that ADMA is not influenced by treatment, but is strongly dependent on smoking, an important finding in the RA population.

5. Independent factors associated with vascular damage were identified: disease duration, disease activity, and type of therapy.

6. It was confirmed that the Framingham Risk Score correlates with ultrasound markers of arterial stiffness and reflects early vascular changes in rheumatoid arthritis.

Practical Contributions

1. It was established that CDAI correlates most accurately with markers of vascular damage, defining its advantage over DAS28-CRP and DAS28-ESR in clinical practice.

2. The key role of disease activity control in limiting arterial stiffness and cardiovascular risk was demonstrated.

3. The need for routine lipid profile monitoring in patients receiving biological therapies and JAK inhibitors was emphasized.

4. The results support individualized cardiovascular risk assessment and guide personalized treatment and follow-up strategies in patients with rheumatoid arthritis.

VIII. List of Publications

1. Dimova-Mileva M, Gerganov GA, Georgiev TA. Impact of the treatment with TNF inhibitors and JAK Inhibitors on arterial stiffness and lipid parameters in rheumatoid arthritis patients: a cross-sectional study. *Eur Heart J Cardiovasc Imaging*. 2025;26(Supplement_1). doi:10.1093/ehjci/jeae333.407
2. Gerganov G, Dimova-Mileva M, Markov M, et al. ABS0086 HOW DISEASE ACTIVITY AFFECTS ULTRASONOGRAPHIC MARKERS OF ARTERIAL STIFFNESS IN RA PATIENTS TREATED WITH TNF INHIBITORS AND JAK INHIBITORS. *Ann Rheum Dis*. 2025;84:1647. doi:10.1016/j.ard.2025.06.1065
3. Gerganov G, Georgiev T, Dimova M, Shivacheva T. Vascular effects of biologic and targeted synthetic antirheumatic drugs approved for rheumatoid arthritis: a systematic review. *Clin Rheumatol*. Published online March 30, 2023. doi:10.1007/s10067-023-06587-8

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I dedicate this work to my father, Alexander, who passed away during my doctoral studies.