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Inclusion of fibrinoid necrosis increases the accuracy of synovial tissue assessment in predicting response to methotrexate: analysis of the UCLouvain Brussels ERA Cohort

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Abstract

Objective Rheumatoid Arthritis (RA) often exhibits suboptimal treatment response despite early diagnosis and treatment. This study aimed to analyze Early Rheumatoid Arthritis (ERA) synovial biopsies through histology and immunohistochemistry (IHC) to identify predictive factors for treatment response to Methotrexate (MTX).

Methods 140 ERA patients from the UCLouvain Arthritis Cohort underwent synovial biopsy and were monitored after initiating Disease-Modifying Antirheumatic Drug (DMARD) therapy. Histological features [Synovial Hyperplasia, Fibrinoid Necrosis (FN), Hypervascularization and Inflammatory Infiltrate] and IHC (CD3, CD20, CD138, CD68) were each semi-quantitatively assessed on a 0–3 scale with 7 levels.

Results A strong association was observed between synovial CD68 and Fibrinoid Necrosis scores [$r = 0.44$ ($0.27 - 0.56$); $p < 0.0001$]. CD68 correlated with C-Reactive Protein (CRP), DAS28, SDAI and CDAI. Fibrinoid Necrosis score correlated with CRP and DAS28. Patients were then categorized as CD68Necrosis^{HIGH} (CD68 + Necrosis ≥ 3) and CD68Necrosis^{LOW} (CD68 + Necrosis < 3). CD68Necrosis^{HIGH} exhibited higher pre-treatment disease activity [5.48 (1.6) versus 4.8 (1.7); $p = 0.03$] and a greater fall in DAS28 [1.99 (2.06) versus 1.1 (2.27), $p = 0.03$], SDAI [21.45 (IQR 23.3) versus 11.65 (IQR 17.5); $p = 0.003$] and CDAI [16 [14.9] versus 10.5 (20.1), $p = 0.04$]. CD68Necrosis^{HIGH} patients had a higher EULAR Moderate/Good Response rate. CD68Necrosis score was incorporated into a probability matrix model together with clinical features (SJC44 and DAS28) to predict achieving a Moderate/Good EULAR Response Criteria at 3 months with a good performance (AUC 0.724).

Conclusion FN and CD68 + in ERA synovial biopsies identify patients with higher disease activity and predict a better treatment response at three months. A model including synovial CD68 and fibrinoid necrosis with baseline clinical features predicts EULAR response at 3 months.

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Introduction

Rheumatoid Arthritis (RA) is a chronic disease primarily presenting with symmetric polyarthritis, leading to joint pain, disability and destruction. Synovitis in RA is characterized by alterations in synovial architecture such as hyperplasia of the synovial lining membrane, hypervascularity and infiltration of the sublining by mononuclear cells. Continuous progress in understanding Rheumatoid Arthritis (RA) pathogenesis led to the development of a number of targeted treatments, including biological Disease Modifying Anti Rheumatic Drugs (bDMARDs) and target sintetic (ts)DMARDs. However, a large number of patients fail to achieve a persistent status of low disease activity or remission. Indeed, the limited good response rate to first-line therapy with cDMARDs means that almost one-half of patients will be eligible for second-line therapy with bDMARDs or tsDMARD [1]. Despite a better knowledge of the disease, we are still not able to predict short and long-term treatment response. The spread out of synovial biopsies, a safe and reliable procedures, opens perspectives of personalized medicine in RA. This was recently illustrated in two biopsy-driven clinical trials [2, 3], investigating response to bDMARD as second or third line therapy, based on synovial pathotypes defined by the presence of B lymphocytes, T lymphocytes, and synovial tissue macrophages (STMs). By contrast, we have recently shown that synovial transcriptomic patterns do not seem to segregate into distinct subgroups, rather correlate with disease activity, mitigating the potential of transcriptomiic profiling to predict therapeutic response [4]. To date, no studies have clearly demonstrated the ability of histology and pathotypes to predict the response to first-line therapy in untreated Early Rheumatoid Arthritis (ERA). Here, we analyzed the ability of a previously understudied histological feature (i.e. fibrinoid necrosis) together with immunohistochemistry evaluation to predict treatment response to methotrexate in a large cohort of untreated ERA.

Materials and methods

One-hundred-forty patients fulfilling the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) Classification Criteria for Rheumatoid Arthritis [5] with symptoms duration and clinical synovitis of less than 12 months duration (Early Rheumatoid Arthritis) were included in the present study as part of the "Early Arthritis UCLouvain Brussels Cohort". All patients were naïve to steroids and DMARDs treatment. Patients underwent needle arthroscopy of the knee or ultrasound (US)-guided synovial biopsy of a clinically involved small joint, prior to starting treatment. The biopsy location was decided, after a systematic US evaluation, according to the presence and degree of synovial hypertrophy as well as the feasibility and safety of the procedure. The standardized pre-biopsy US-scanning included: metacarpophalangeal joints (MCPs), Wrists, Knees, metatarsophalangeal joints (MTPs), and elbows. At least 3 fragments for each patient were collected for histological evaluation. The procedure and the tissue analysis were performed according to the EULAR minimal report requirements for synovial tissue research [6].

A standard cDMARDs therapy (±a low corticosteroid dose) was prescribed, with a treat-to-target approach according to international guidelines [<3.2 Disease Activity Score-28 ($DAS28_{CRP}$) as target]. In non-responder patients to cDMARDs therapy, a bDMARDs therapy was added to treatment. Twelve patients were treated with bDMARDs (TNFi or anti-IL-6) as first-line therapy alone or in combination with cDMARDs: these patients were thus excluded, and one-hundred-twenty-six patients were evaluated for the longitudinal analysis of Methotrexate (MTX) treatment response. Follow-up visits were programmed each 1–3 months during active disease until the remission or Low Disease Activity LDA was achieved and then every 4–6 months. Radiographs of hands and feet were obtained at baseline to assess the presence of bone erosion.

Histological and immunohistochemical evaluation

Tissue samples were fixed overnight in 10% formalin buffer at pH 7.0 and embedded in paraffin for histology and immunochemistry. Four parameters were assessed on Hematoxylin Eosin (H&E) slide: 1) degree of Lining Layer Hyperplasia, scored according to the increase in the number of lining cell layers (with/without multinucleated cells); 2) intensity of inflammatory infiltrate, according to the presence, abundance, and distribution of inflammatory infiltrate (including the presence of lymphocytes and follicle-like aggregates; 3) Fibrinoid Necrosis (FN), defined as the presence of fibrin deposition within and around the capillaries and/or small vessels; 4) Hypervascularization, evaluated as an increased number and/or size of vascular cross-sections per analyzed section. Each parameter was scored on a semiquantitative scale with 7 levels [0–3] by an expert pathologist blinded to clinical data (CG).

Similarly, with the same scoring system [0–3], the degree of immune cell infiltration was determined through immunohistochemical staining for B cells (CD20) [CD20 Biocare Medical Clone L26, dilution 1:200], T cells (CD3) [CD3 Roche Clone 2GV6], Macrophages (CD68) [CD68, Dako clone PGM1 1:40], and Plasma Cells (CD138) [CD138 Dako Clone M&15 1:20].

In a separate analysis, our synovial samples were re-classified according to the classic pathotypes classification [7, 8]. With this categorization, based on a semiquantitative assessment [0–4 scale] of immune cells infiltration, biopsies are stratified in three pathotypes

according to these criteria: Lympho-myeloid (LM) presence of grades 2–3—CD20+aggregates, (CD20≥2) and/or CD138≥2; Diffuse-myeloid (DM): CD68≥2, CD20≤1 and/or CD3≥1, CD138≤2 and Pauci-immune-fibroid (PI): CD68<2 and CD3, CD20, CD138<1. However, considering that our histological evaluation is based on a different scoring system [0–3 scale], we have to adapt the pathotype classification as follows: Lympho-myeloid (LM): presence of grade 2–3 CD20+aggregates, (CD20≥2) and/or CD138; Diffuse-myeloid (DM): CD68 sublining (SL)>1, CD20≤1 and/or CD3≥1, CD138≤2; Pauci-immune (PI): CD68≤1 and CD3, CD20, CD138<1.

Statistical analysis

All analyses were performed with SPSS 26.0.0 and GraphPad 9.0. Normally distributed variables were summarized using the mean (SD), and non-normally distributed variables by the median and range (IQR). Frequencies were expressed by percentage. Wilcoxon's matched pairs test and paired t-test were performed accordingly. Univariate comparisons between nominal variables were calculated using the chi-square test or Fisher's exact test where appropriate. Spearman's test was used to assess the correlations. To assess positive predictive value (PPV), negative predictive value (NPV), specificity and sensitivity, the Wilson-Brown method was applied. Two-tailed *p* values were reported, and *p* values less than 0.05 were considered significant.

Table 1 Characteristics of the whole cohort

	Median (IQR) / *N*° (%)
Age (years)	52.7 (21.4)
Female/Male	105/35 (75%/35%)
Time to Biopsy (Weeks)	6 (9)
TJC (28)	6 (8.5)
SJC (28)	6 (8)
TJC (44)	7 (10)
SJC (44)	7 (10)
CRP (mg/dL)	1.59 (3.3)
HAQ	1.5 (1.125)
DAS 28	5.19 (1.73)
SDAI	28.8 (20.1)
CDAI	26 (19.25)
VASm	4 (2)
VASp	7 (3.1)
Rheumatoid Factor	51.1%
anti-CCP	52.6%
Methotrexate (Weekly Dose)	20 mg (5)
Methotrexate N° Pts (%)	126 (90%)
Glucocorticoids N ° Pts (%)	25 (17.8%)
Glucocorticoids (PDN Daily Dose)	5 mg (1.25)
Salazopyrin N° (%)	1 (0.7%)
anti-IL6R N° (%)	10 (8.2%)
anti-TNF N° (%)	2 (1.4%)
Biopsy Location N° (%)	
Knee	54 (38.6%)
Wrist	46 (32.9%)
PIP	2 (1.4%)
MCP	27 (19.6%)
MTP	10 (7.1%)
Elbow	1 (0.7%)

BMI=Body Mass Index; TJC=Tender Joint Count; SJC=Swollen Joint Count; CRP=C-Reactive Protein; HAQ=Health Assessment Questionnaire; DAS 28=Disease Activity Score 28 Joint; SDAI=Serological Disease Activity Index; CDAI=Clinical Disease Activity Index; MCP=Metacarpophalangeal; PIP=Proximal Interphalangeal; MTP=Metatarsophalangeal; PDN=Prednisone

Predictive matrix

To determine the predictors of reaching EULAR Response Criteria a two-step analysis was performed. First, in univariate analysis, all baseline variables were tested to establish the statistical significance of the relationship between candidate predictors and a Moderate/Good EULAR Response. Each variable for which the *p*-value was below 0.10 was chosen as a potential predictor and was then included in the second step. For each variable, the cut-off was chosen according to the 1st and the 3rd quartiles given by the distribution (Supplementary). Second, multivariate logistic regression analysis with EULAR Response Criteria (Moderate or Good) as the dependent variable was performed using all the possible combinations of predictors selected in the first step. The overall discrimination power of the model was evaluated by receiver operating characteristic curve (ROC) analysis and the calculation of the area under the ROC curve (AUC). The fit of the model was assessed by the Hosmer-Lemeshow test.

Results

Cohort baseline features

One-hundred-forty patients were included [Female/Male (F/M) 35/105; Median age 52.7 years (IQR 21.4)] with a median time from onset of symptoms and synovial biopsy of 6 weeks (IQR 9). The baseline features of the cohort are reported in Table 1. Briefly, the median DAS28 was 5.19 (IQR 1.73), with seropositivity for Rheumatoid Factor and anti-CCP of 51.1% and 52.6% respectively.

All patients were naïve to therapy (DMARDs and/or glucocorticoid) at the moment of the biopsy. After the procedure, one-hundred-twenty-six patients (90%) were treated with a median MTX dose of 20 mg (IQR 5); only twenty-five patients received an initial glucocorticoid therapy with a median daily prednisone (PDN) dosage of 5 mg (IQR 1.25). The most representative biopsy sites were knee and wrist (38.6% and 32.9% respectively).

Histological and baseline disease features

We first assessed the relationship between the histological features according to the IHC and H&E scores. As represented in the bubble plots (Fig. 1A-D), CD3

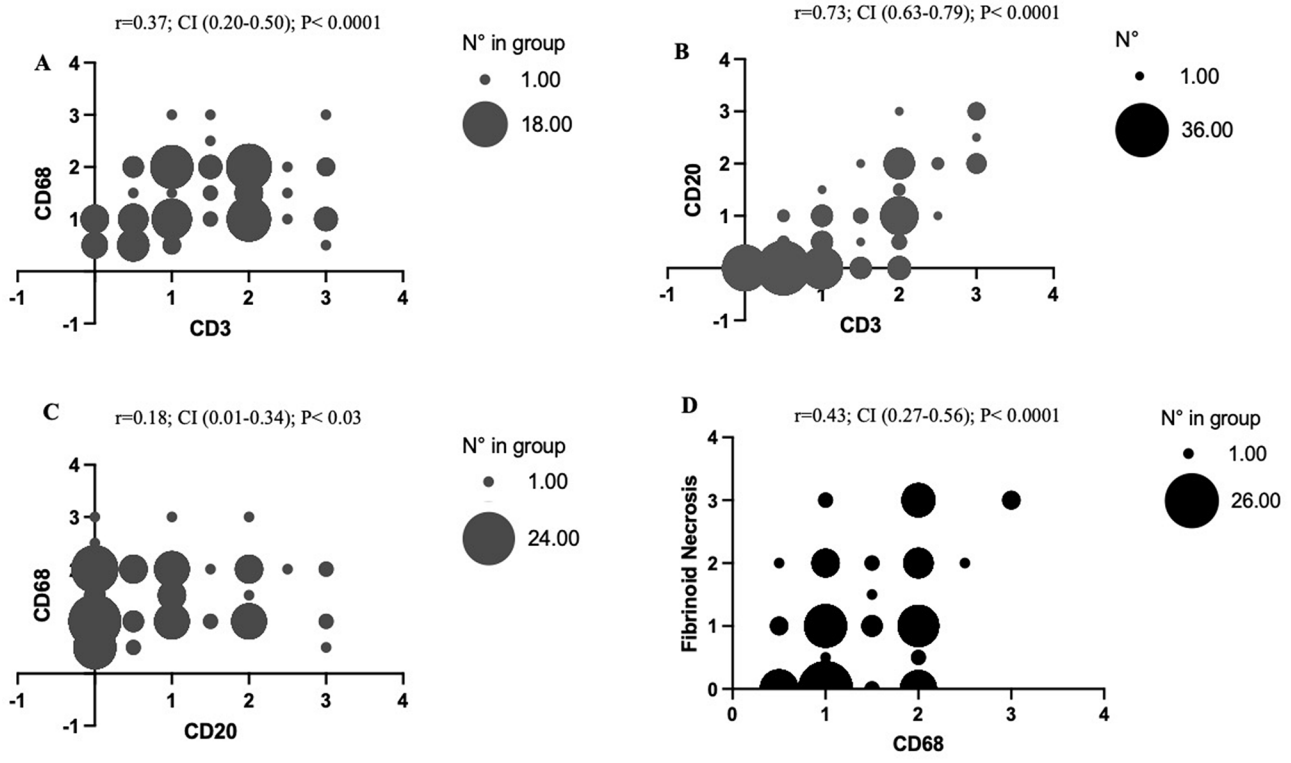


Fig. 1 (**A**-**D**). Bubble plot representing IHC and Histological score distribution. Each Graph represents a couple of combined scores: **A** = CD3-CD68; **B** = CD3-CD20; **C** = CD20-CD68; **D** = CD68-Fibrinoid Necrosis; the number of patients for each group (i.e., CD3score = 1 and CD68 = 1) is represented by bubble size

infiltrate showed a good correlation with CD68 (r=0.37, p<0.0001) and CD20 (r=0.73, p<0.0001). More interestingly, macrophages were also strongly associated with Fibrinoid Necrosis (r=0.43, p<0,0001) stronger than with any other variable analyzed.

We then assessed the relationship between histological or IHC features and the baseline clinical characteristics of the cohort. Both CD68 and Fibrinoid Necrosis scores were associated with baseline CRP (r=0.31; p=0.001 and r=0.29; p=0.001 respectively), and DAS28 (r=0.26; p=0.005 and r=0.26; p=0.009 respectively). Moreover, the CD68 score was positively associated with SDAI (r=0.28; p=0.008), CDAI (r=0.25; p=0.018). Among all the items analyzed, CD68 and Fibrinoid Necrosis were the histological scores showing the best positive correlations with baseline clinical indexes and C-Reactive Protein (Supplementary Fig. 1, Supplementary Table 1).

Tissue samples were also categorized according to pathotype classification (10). Forty-three patients (30.7%) matched the Pauci-Immune (PI) pathotype, while the remaining ninety-seven (69.3%) were characterized by a more inflammatory phenotype, resembling Diffuse Myeloid (DM) (37.1%) and Lympho-Myeloid (LM) pathotype (32.2%). Baseline clinical and disease features are summarized in Supplementary Table 2.

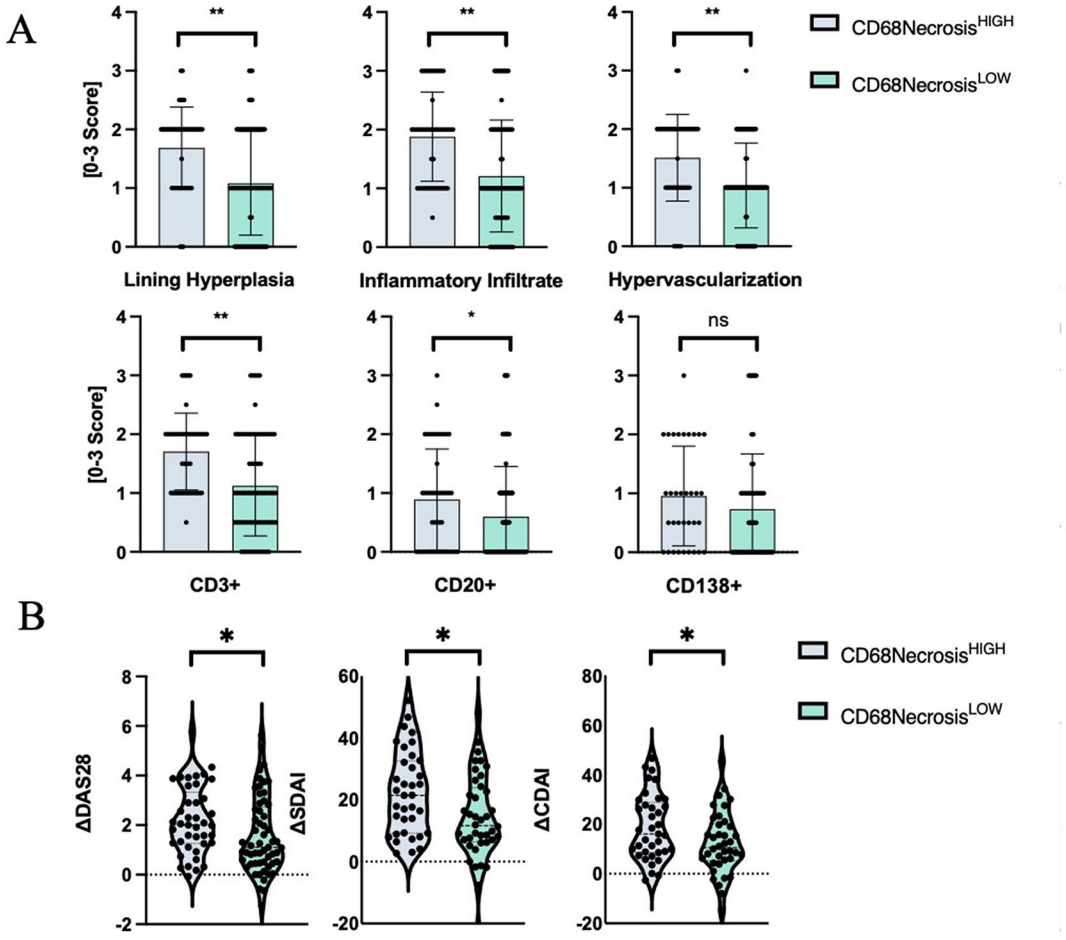
CD68Necrosis score

Considering the association identified between IHC and Histological features and clinical characteristics, we created a semiquantitative score combining the two semiquantitative scores (CD68 + Fibrinoid Necrosis):

$$\text{CD68Necrosis Score} = \text{CD68 Score}\,[0-3] + \text{Necrosis Score}\,[0-3]$$

Applying this definition, patients were divided into two groups: **CD68Necrosis^{HIGH} ≥ 3** and **CD68Necrosis^{LOW} < 3** (43 and 72 patients respectively). Tissue samples and patients' features were re-analyzed, comparing the two groups. From a histological point of view, CD68Necrosis^{HIGH} patients compared to CD68Necrosis^{LOW} were distinguished by higher inflammatory histological features [Lining Hyperplasia (1.68±0.69 versus 1.08±0.88);p=0.0002), Inflammatory Infiltrate (1.88±0.76 versus 1.21±0.95; p<0.0001) Hypervascularization (1.51±0.74 versus 1.04±0.72; p<0.0003), and a higher immunostaining score for CD3 (1.70±0.65 versus 1.12±0.85 p<0.0001and CD20 (0.89±0.85 versus 0.59±0.84;p=0.04) (Fig. 2A).

Regarding clinical features, CD68Necrosis^{HIGH} was characterized by a more inflammatory phenotype with higher CRP (2.5 mg/dL [IQR 4] versus 0.92 mg/dL [IQR 2.95]; p=0.02), SDAI (33.1 [IQR 17.4] versus 25.6 [IQR



C

	ΔDAS28	Δ SDAI	ΔCDAI	ΔDAS28	Δ SDAI	Δ CDAI	Δ DAS28	Δ SDAI	Δ CDAI
CD68Necrosis^{HIGH}	2.18 (1.36)	22.2 (13.5)	18.7 (13.1)	2,75 (1.66)	25. (15.4)	22,55 (14.2)	2,5 (1.7)	27,1 (16.8)	23,75 (15)
CD68Necrosis^{LOW}	1.58 (14.1)	14.1(13.8)	12.3 (12.4)	2.2 (1.46)	17.7 (13.4)	17.7 (12.1)	2.32 (1.64)	19.8 (12.6)	19 (11.9)
p	0.03	0.04	0.003	0.19	0.07	0.22	0.54	0.03	0.09

Fig. 2 Comparison of histological evaluation and treatment response between CD68Necrosis^{HIGH} and CD68Necrosis^{LOW}

21.44]; $p=0.03$) and DAS28 (5.48 [IQR 1.69] versus 4.8 [IQR 1.74]; $p=0.03$), while no significative difference emerged regarding any other clinical features. The number of TJS and SJC did not differ between the two groups (Table 2).

Three-months methotrexate treatment response

Each patient was evaluated for treatment response every three months and up to one-year follow-up. At three months of evaluation, CD68Necrosis^{HIGH} had a better treatment response when evaluated with ΔDAS28, ΔCDAI, and ΔSDAI (Fig. 2B). The difference did not reach statistical significance from 6 months onwards for none of the studied indexes (Fig. 2C).

CD68Necrosis score predicts EULAR Response at 3 months

To account for the difference in baseline DAS28 between the two groups (5.48 [1.69] *versus* 4.8 [1.74]), we applied EULAR Response Criteria definitions, to evaluate the reliability of the CD68Necrosis score in predicting the achievement of an EULAR good/moderate response. In line with previous results, patients with a CD68Necrosis^{HIGH} were more prone to reach at least a Moderate EULAR Response compared to CD68Necrosis^{LOW} patients at three months (90% versus 71.43%, OR=3.6 [1.18–10.68]; $p=0.036$, PPV=0.9 (0.76–0.96), NPV=0.28 [0.17–0.41]. Figure 3A).

Probability Matrix

We explored the capacity of our classification to identify the probability of achieving a Moderate/Good Response for a single patient combining the CD68Necrosis score

Table 2 Baseline clinical features of the two derived cohorts CD68Necrosis^{HIGH} CD68Necrosis^{LOW}

	CD68Necrosis^{HIGH}	CD68Necrosis^{LOW}	
N° Patients	43 (37.4%)	72 (62.6%)	
(IQR)			
TJC (28)	6 (8)	5 (8.5)	0.69
SJC (28)	8 (7)	5 (8.5)	0.16
HAQ	1.75 (1.06)	1.5 (1.21)	0.19
DAS28	5.48 (1.69)	4.8 (1.74)	0.03
CRP	2.5 (4)	0.92 (2.95)	0.02
CDAI	29.25 (14.35)	23.85 (17.93)	0.25
SDAI	33.10 (17.4)	25.65 (21.44)	0.03
Age (Years)	56.4 (25.6)	48.32 (22.1)	0.08
Time to Biopsy	4 (9)	6 (10.75)	0.33
BMI	21.35 (7.97)	20.10 (4.95)	0.30
(%)			
RF	60.3	47.8	0.16
ACPA	58.4	49.2	0.31
Erosions	55.5	50.7	0.70
Smokers	18.6	19.6	0.99

BMI=Body Mass Index; TJC=Tender Joint Count; SJC=Swollen Joint Count; CRP=C-Reactive Protein; HAQ=Health Assessment Questionnaire; DAS 28=Disease Activity Score 28 Joint; SDAI=Serological Disease Activity Index; CDAI=Clinical Disease Activity Index; RF=Rheumatoid Factor; ACPA=anti citrullinated proteins antibodies

with the discriminating baseline features of the two groups. This approach more realistically reproduces the daily clinical practice, by combining the disease activity evaluation of a patient (including clinical and laboratory features), along with performing a CD68/FN score on a synovial biopsy. The variables associated with a better response to univariate analysis have been included in a multivariate logistic regression model to identify each patient's probability of reaching a Moderate/Good Response (Supplementary Tables 3 and Supplementary Fig. 2). The cut-off values for each variable have been set according to the 25th and 75th percentile (Supplementary file). According to the Area Under the Curve (AUC) ROC the best-performing model was chosen, including as variables CD68Necrosis score, SJC (44), and DAS28 (AUC=0.724; Std. Error 0.072 [95%CI 0.58–0.86]; p=0.0028). The graphical representation of the Matrix is represented in Fig. 3B.

Remission criteria

When applying different remission criteria (DAS28, Boolean 2.0, and SDAI) no differences were identified

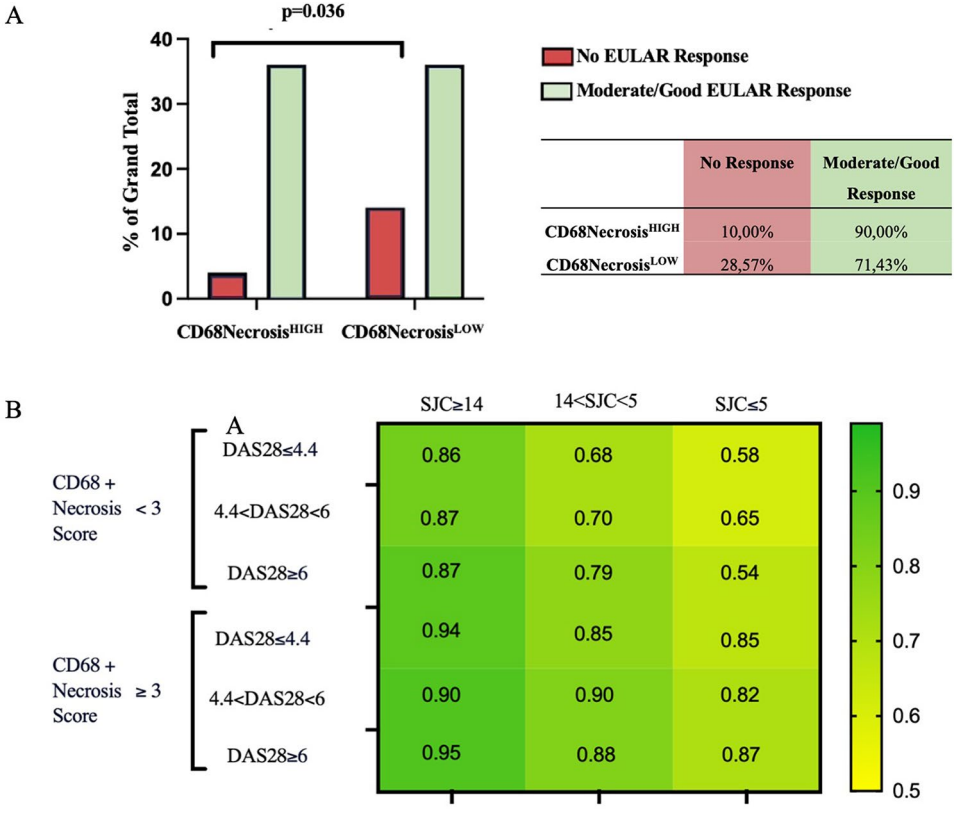


Fig. 3 Three months methotrexate treatment response. **A**) Comparison of Moderate/Good Response Achievement at Three Months of follow-up between CD68Necrosis^{HIGH}*versus* CD68Necrosis^{LOW} patient**s** B**)** Three Months EULAR Moderate/Good Response Probability Prediction Matrix: the values reported express the 3 months probability of No EULAR Response [0–1 = 0 – 100%] for each combination of included categorized variables. For example, a patient with CD68 + Necrosis Score < 3, SJC ≥ 14 and DAS28 ≥ 6 has a 3-month probability of reaching at least a Moderate EULAR Response of 87%

between the two groups up to 12 months of follow-up (Table 3).

Long-term treatment strategy

In a long follow-up period of up to 36 months, we investigated any differences in treatment strategy including cDMARDs combined therapy [MTX plus Salazopyrin (SSZ) or Hydroxychloroquine] or any bDMARDs/tsDMARDs. The treatment strategy was similar in both groups, with neither of the two groups (CD68Necrosis^{HIGH} and CD68Necrosis^{LOW}) requiring a higher number of bDMARD/tsDMARDs or combined therapy prescriptions (Supplementary Table 4).

Discussion

In our study, we present the evidence that histological assessment can serve as a predictive tool for treatment response in ERA patients naïve to any DMARD. Our scoring system, derived from a combination of Macrophages and Fibrinoid Necrosis histological semi-quantitative assessments, successfully identifies a subset of ERA patients (referred to as CD68Necrosis^{HIGH}) within a large cohort. These patients exhibit a more inflammatory phenotype at baseline and experience a greater reduction in DAS28, SDAI, and CDAI when treated with MTX. This finding was further replicated when using (EULAR) Response Criteria.

Our study builds upon previous efforts in recent years. In the PEAC cohort of untreated ERA patients, Humby and colleagues [8] examined the 6-month treatment response to conventional cDMARDs with MTX being the predominant treatment (utilized in 90% of cases) applying the pathotypes classification (PI, LM, and DM). However, histology alone was not sufficient to predict treatment response based on DAS28ESR or EULAR Response Criteria in their study.

By contrast, our study successfully predicted treatment response solely through histological assessment. This discrepancy in results can be attributed to differences in histology-based subgroup classification and additional histological evaluation such as Fibrinoid Necrosis.

The latter has never been taken into consideration in other publications as a predictor or biomarker of disease severity. FN is a type of tissue necrosis characterized by the deposition of fibrin-like material and cell death [9] and, by its nature, can only be identified through histology. The specific mechanisms of FN development are still unclear, and the relative literature is scarce. Specific studies on rheumatoid nodules (of which it is the histological hallmark) demonstrated a macrophage-mediated origin through the complement activation [10]. It is a common histological feature to various autoimmune diseases (i.e. in ANCA vasculitis) [9] and, whatever is the initial trigger, it leads to cell damage, accumulation of serum protein and thus fibrin polymerization.

The presence of FN in RA synovium is known, but it has never been systematically studied: only one previous descriptive paper reports FN in almost 70% of RA synovial biopsies [11]. On the contrary, synovial macrophages have already shown a promising role as biomarkers of therapy response. In previous repeated biopsy protocol studies, synovial CD68 depletion from baseline correlated with improved treatment outcomes to various medications (cDMARDs, TNFi, Abatacept, Rituximab) [12–17] and similarly their failure to decrease after therapy has been proposed as biomarker of poor treatment response [18–20]. All these data suggest that synovial macrophages may be a reliable biomarker of treatment response in RA, independently on the mechanism of action of the treatment.

Although the score demonstrated a good performance from a predictive point of view, the relationship between the macrophage infiltrate and the abundance of fibrinoid necrosis remains elusive; the latter can accumulate over time and reflect the inflammatory process both present and past. Although both demonstrated a close association with each other and with inflammation laboratory and clinical variables, further studies should confirm this association. Moreover, despite its affordability and reproducibility, immunohistochemistry (IHC) may not consistently possess the required sensitivity to pinpoint the most optimal responders, particularly in smaller patient cohorts. Therefore, molecular experiments were performed to overcome the intrinsic method limits, and pre-treatment myeloid gene expression in RA synovium has effectively demonstrated the ability to predict response to TNFi [21] and cDMARDs [22] as to be substantially downregulated by various DMARDs [17].

The CD68Necrosis score was also included in a predictive algorithm, which ended in the presented probability matrix. This tool has been already tested as support to clinicians in determining the single-patient risk of

Table 3 Percentage of Remission (DAS8, Boolean 2.0 and SDAI) achievement in CD68Necrosis^{HIGH} and CD68Necrosis^{LOW} at three, six, and twelve months of follow-up

	T3			T6			T12		
	DAS28	**Boolean 2.0**	**SDAI**	**DAS28**	**Boolean 2.0**	**SDAI**	**DAS28**	**Boolean 2.0**	**SDAI**
CD68Necrosis^{HIGH}	32.6/67.4	22.5/77.5	21.4/78.6	63.4/36.6	32.6/67.3	40.5/59.5	51.3/48.7	27.6/72.3	29/71
CD68Necrosis^{LOW}	31.5/68.5	17.6/82.4	26/74	61.1/38.9	19.7/80.3	39.6/60.4	62.5/37.5	28.1/71.9	39.1/60.9
p	0.99	0.64	0.63	0.83	0.13	0.99	0.29	0.99	0.46

achievement of a specific outcome in RA, for instance, the risk of 1-year radiographic progression in ERA [23]. As already shown by Alivernini and colleagues [24], synovial tissue analysis could represent an added value in the development of a patient-features-centered algorithm, aiming at predicting which patients are more prone to reach remission with a specific treatment. For the first time we were able to merge baseline clinical (DAS28 and SJC44), and histology features (CD68Necrosis Score) to define the pre-treatment (MTX) chance of an ERA cohort to reach a Moderate/Good EULAR.

Our study has several strengths and some limitations. We evaluated a large single-center cohort of ERA naïve to any DMARDs and among them, a subgroup exclusively treated with MTX. Only a low percentage of patients received low-dose glucocorticoids, ensuring a uniform clinical evaluation and management. Moreover, the short time between the onset of symptoms and synovial biopsies is a guarantee of high-quality tissue analysis in terms of medication effect on histology. In addition, we show the usefulness of the histological in the very first months after diagnosis and treatment begins, the critical period in the hypothesis of the 'window of opportunity". On the other side, the non-controlled observational design of the study and the heterogeneity of the medications (in terms of csDMARD and/or b/tsDMARDs dose and combination) from three months onwards could have influenced the analysis and outcomes, explaining the limited usefulness in a mid-long-term observation of our score.

The main limitation of this study is related to the incapacity to better characterize the macrophage infiltrate and the absence of transcriptomic analysis. Indeed, we have recently shown that M1-like (CD68+CD206-) or M-2 like (CD68+CD206+) synovial macrophages in the lining layer are differently associated with synovial inflammatory burden and clinical disease activity [8].

Conclusion

In conclusion, the evaluation of Macrophages and Fibrinoid Necrosis in ERA synovial biopsies identifies patients with higher disease activity and with a better DAS28 fall at three months. The semiquantitative score can predict the achievement of EULAR Response Criteria, alone or with clinical baseline features.

Abbreviations

RA	Rheumatoid Arthritis
DMARDs	Disease Modifying Anti Rheumatic Drugs
cDMARDs	classic DMARDs
bDMARDs	biological DMARDs
tsDMARDs	target synthetic DMARDs
STM	Synovial tissue macrophages
ERA	early rheumatoid arthritis
ACR	American College of Rheumatology
EULAR	European League Against Rheumatism
US	ultrasound
MCPs	metacarpophalangeal joints
MTPs	metatarsophalangeal joints
TNFi	Tumor Necrosis Factor Inhibitor
MTX	Methotrexate
LDA	Low Disease Activity
H&E	Hematoxylin Eosin
FN	Fibrinoid Necrosis
IQR	interquantile range
PPV	positive predictive value
NPV	negative predictive value
ROC	receiver operating characteristic curve
AUC	area under the ROC curve
PDN	prednisone
BMI	Body Mass Index
TJC	Tender Joint Count
SJC	Swollen Joint Count
CRP	C-Reactive Protein
HAQ	Health Assessment Questionnaire
DAS 28	Disease Activity Score 28 Joint
SDAI	Serological Disease Activity Index
CDAI	Clinical Disease Activity Index
PI	Pauci-Immune
DM	Diffuse Myeloid
LM	Lympho-Myeloid
RF	Rheumatoid Factor
ACPA	anti-citrullinated proteins antibodies
SSZ	Salazopyrin
IHC	immunohistochemistry

Supplementary Information

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Supplementary Material 1

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Author contributions

F.N. and P.D. wrote the main manuscriptF.N., C.T., C.VM., T.S., E.S., L.M., A.N., C.G., B.L., and P.D. t analysed the data, prepared the figures and tables and contributed to the drafting of the manuscript.All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted according to the Declaration of Helsinki. All patients provided written informed consent.

Consent for publication

Not applicable.

Competing interests

B. Lauwerys is a full-time employee and stockholder of UCB Pharma.

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