Major vault protein/lung resistance related protein: a novel biomarker for rheumatoid arthritis

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Abstract

Objective

Rheumatoid arthritis (RA) can lead to joint destruction and early institution of effective treatment can preserve joint function. Biomarkers can establish early diagnosis and predict effect of treatment. Vault particles, large cytoplasmic ribonucleoprotein particles that participate in inflammation, might serve as biomarkers. The aim of this study was to assess the diagnostic and the prognostic value of major vault protein (MVP) and their antibodies in RA.

Methods

Serum samples from 159 RA patients, 26 early RA (ERA) patients, 21 patients with osteoarthritis (OA) and 30 healthy individuals were tested for MVP, anti-cyclic citrullinated peptide (anti-CCP) and C-reactive protein (CRP) using enzyme-linked immunosorbent assays (ELISA). Rheumatoid factor (RF) was tested by nephelometry, and anti-MVP antibodies were detected by anti-MVP peptide ELISA using an in-house protocol.

Results

MVP levels were higher in RA and ERA, compared to OA and healthy controls ($p<0.00001$). A combination of MVP with RF or anti-CCP showed an improved diagnostic accuracy compared to RF or anti-CCP alone in RA and ERA. MVP exhibited similar AUC levels to anti-CCP and RF in RA whereas in ERA, MVP exhibited the same or slightly higher AUC levels, compared to anti-CCP and RF, respectively. High MVP levels were associated with lack of response to treatment. Levels of anti-MVP peptide 2 antibodies were significantly higher in RA compared to healthy controls ($t= 2.73$, $p=0.007$).

Conclusion

MVP and autoantibodies against MVP may have the potential to serve as diagnostic and prognostic biomarkers in RA.

Key words
rheumatoid arthritis, biomarkers, autoantibodies, major vault protein, lung resistance related protein

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Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease (1, 2). Early diagnosis of RA and early institution of effective treatment are crucial for symptomatic relief and prevention of joint damage, ensuring a relatively good quality of life for the patient (3). Thus, the discovery of new biomarkers to achieve an early diagnosis or to predict response to treatment is of high importance.

Described for the first time in 1986 (4) vaults are the largest ribonucleoprotein particles in the cytoplasm of eukaryotic cells (5) and consist of three proteins; major vault protein (MVP) (96 kDa); vault poly (ADP-ribose) polymerase (VPARP) (193 kDa); telomerase-associated protein 1 (TEP1) (290 kDa); and small, untranslated RNA (vRNA). MVP accounts for more than 70% of their mass (Fig. 1A). Although their function is largely unknown, several researchers have suggested a role for vaults in cancer cells' ability to resist chemotherapy. MVP also has a recently identified role in the regulatory mechanisms of inflammatory response. It has been demonstrated that MVP interacts with TRAF6 and suppresses the activation of the IKK-NF-κB signal pathway (6).

Vaults are related to autoantigens commonly targeted in rheumatic diseases and especially to Ro and La autoantigens (7). Interestingly, the Ro autoantigen possesses the TROVE (Telomerase, Ro, and Vault Element) molecular module that shares high sequence similarity with the TEP1 component of the vaults (8). Moreover, it was found that vaults interact specifically the with La autoantigen. The vault RNA complexes with the La autoantigen forming a separate smaller ribonucleoprotein particle in the interior of the vault structure. (4, 9). The goal of this study was to determine whether MVP has the potential to serve as a novel diagnostic marker in RA and ERA. Additionally, existing correlations between MVP levels and disease activity indices, as well as the type of treatment given were also examined.

Materials and methods

Patients and sera

This study approved by the local Ethics Committee in Research. Sera from patients with RA, ERA, OA and healthy controls were obtained from the Department of Rheumatology and clinical immunology, University General Hospital of Larissa and Naval Hospital of Athens during their standard diagnostic or follow-up procedures. In total, we studied sera for 185 RA patients diagnosed according to the American College of Rheumatology criteria (10). Twenty-six of these patients were classified as ERA (<6 months of disease duration). In addition, sera for 21 patients with Osteoarthritis (OA) and 30 healthy individuals served as controls.

Disease activity indices

Disease activity and severity of RA were tested using tender joint count (TJC, 0–28 score), swollen joint count (SJC, 0–28 score), CRP level (mg/dl) and visual analogue scale (VAS) for pain (0–10 cm) as well as disease activity score (DAS28-CRP score, We also used Health Assessment Questionnaire Disability Index (HAQ-DI score). Radiographic assessment was scored using Larsen score. The total score ranged from 0–160 (11). All these scores were evaluated at the time of presentation of patients with RA, as well as two years after.

MVP, RF, CRP, a-CCP quantification

MVP quantification was performed using an MVP sandwich Elisa assay (Novateinbio, Hölzel Diagnostika GmbH, Köln, Germany, NB-E11592 kit). Anti-CCP and RF were measured by commercial ELISA assays (third generation assay 3.1 Inova Diagnostics), while CRP was assessed by nephelometry (Dade Behring BNII nephelometer, positivity limit = 0.5 mg/dl). Since no established cut-off value for serum MVP was established so far, we performed a ROC analysis in conjunction with Youden Index to estimate optimal cut-off values.

Soluble peptides

To identify autoantibodies against MVP, peptide synthesis was carried out. Two selected peptides, MVP-pep1 ($[NH_2$-CWDRDGKERVTGEEWLVTTVG$]_8$-$[K]_7$-βAla-COOH), MVP-pep2 ($[NH_2$-LAYNWHFEVNDRKDPQETAKL$]_8$-$[K]_7$-βAla-COOH), were synthesised

by solid-phase synthesis (Biosynthesis Inc., Lewisville, TX) (Fig. 1B). Both epitopes were synthesised in the form of peptide dendrimers (multiple antigenic peptides, MAPs). Each peptide was purified using reversed-phase high-performance liquid chromatography and was found to exhibit a single peak at its predicted molecular weight measured by mass spectrometry.

Anti-MVP peptide antibody ELISA

Briefly, high-binding microtitre plates (Costar, Corning Life Sciences, Acton, MA) were coated in duplicates with the MVP-pep1 or MVP-pep2 at concentrations of 1 μg/ml and 20 μg/ml respectively in acetate buffer 0.1M (pH=4.0). The plates were incubated for 2 hours, and then plates were washed with phosphate buffered saline (PBS) once and blocked with PBS containing 2% bovine serum albumin (BSA) at RT for 1 h. The plates were washed 3 times with PBS, and sera in a 1:50 dilution containing 2% BSA were incubated at 4ºC overnight. After incubation plates were washed with PBS 3 times, and incubated with alkaline phosphatase conjugated donkey anti-rabbit IgG (H+L) antibody (Thermo Scientific, Waltham, MA), diluted in 2% BSA at RT for 1 h. Then plates were washed with PBS 4 times, and p-nitrophenyl phosphate (pNPP) substrate in substrate buffer was added to detect antibody binding. OD was measured at 405 nm on an ELISA plate reader (Chromate reader; Awareness Technology, Palm City, FL). Cut-off was set at two standard deviations above the average (mean) OD of the normal sera.

Statistical analyses

Pearson's test, Chi-square test, Student's *t*-test were performed using GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla, Ca). Receiver operating characteristic (ROC) analysis was carried out using the SPSS v. 19.0 software (SPSS Inc, Chicago, IL). The optimal cut-off was identified using the Youden index.

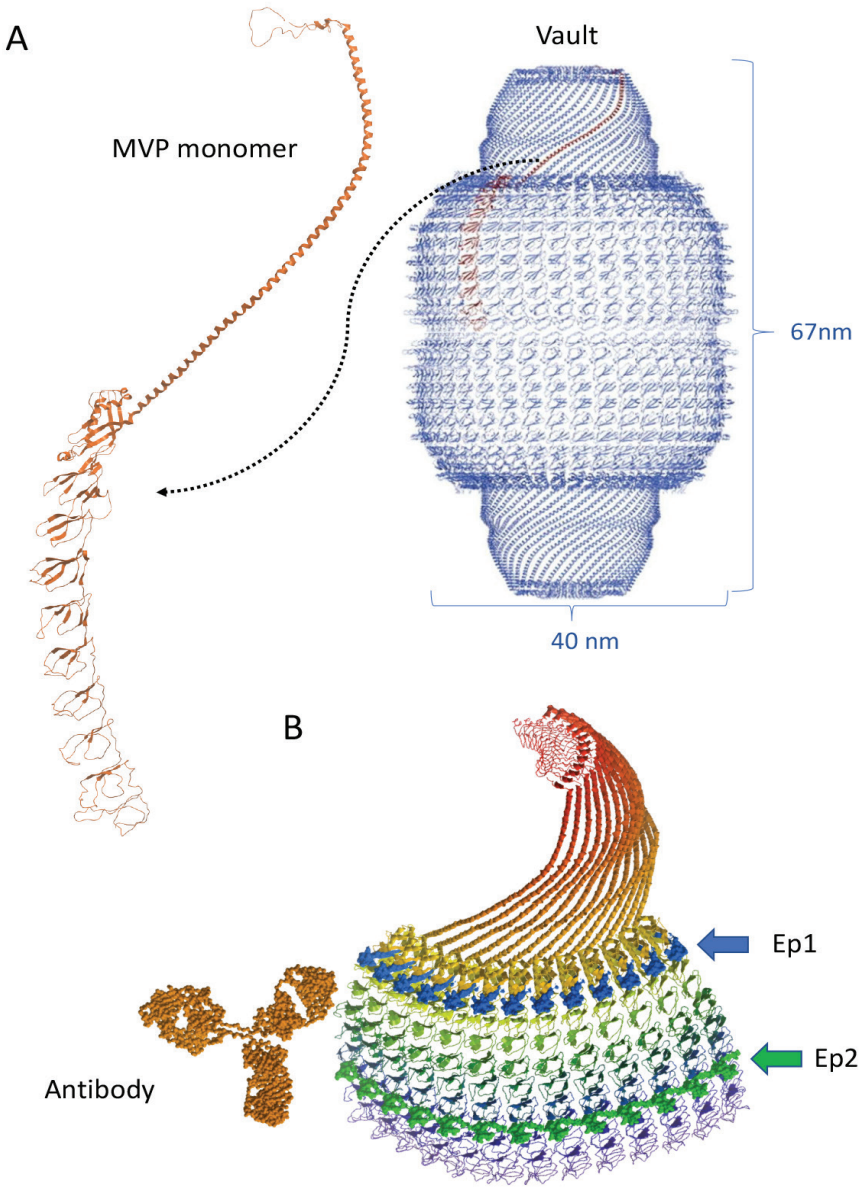

Fig. 1. Vault structure and localisation of selected epitopes in the MVP protein.
A: The structure of vault particle and scheme of MVP configuration within the vault structure.
B: Two surface epitopes in the MVP, epitope 1 (Ep1) and epitope 2 (Ep2) were predicted on the basis of their antigenicity (Welling, Hopp/Woods and Parker indices) and their exposure in the 3D structure of MVP (PDB ID: 4V60).

Results

Characteristics of patients and controls

A total of 185 patients with RA/ERA and 51 controls were included in the study. Their demographic, serological and disease activity characteristics are presented in Table I.

Anti-CCP was found positive in 60.5% (112 of 185) of the RA/ERA cases and in 1.96% (1 of 51) of the controls, whereas RF was found to be positive in 57.3% (106 of 185) of the RA/ERA cases and in 3.9% (2 of 51) of the controls.

Patients with ERA and RA show higher levels of MVP compared to healthy individuals and OA patients

MVP was measured in 236 samples. One hundred and three of the RA patients (55.7%) and sixteen out of twenty-six ERA patients (61.5%) were positive for MVP, whereas only 6 out of 51 control sera (11,76%) and four out of twenty-one (19.05%) of OA patients were MVP-positive. A significant correlation between MVP positivity and ERA or RA was observed (in ERA: χ^2=20.91; p<0.00001; in RA: χ^2=31.01; p<0.00001).

MVP levels were found to be elevated in ERA and RA patients compared to healthy individuals (t=3.96; p=0.0002

Table I. Features of the study population.

Variables	RA patients	ERA patients	Disease Controls	Healthy Individuals
Number (n)	159	26	21	30
Age (years) ± SD	62 ± 12	65 ± 14	63 ± 16	50 ± 17
Female / Male	121/ 38	14/ 12	18/ 3	23/ 7
CCP positive	62.9%	42.3%	0%	3.3%
RF positive	59.7%	42.3%	4.8%	3.3%
CRP positive	41.5%	92.3%	19.1%	0%
DAS28 ± SEM	4.7 ± 0.2	6.4 ± 0.2	NA	NA
Duration of disease ± SEM	115.7 ± 8.4	< 6 months	NA	NA
Tender joint count ± SEM	8.8 ± 0.8	3.3 ± 1.1	NA	NA
Swollen joint count ± SEM	5.1 ± 0.7	1.6 ± 0.4	NA	NA

NA: not applicable.

Table II. MVP status in relation to anti-CCP antibodies and RF.

	RA patients n=185	Controls n=51
MVP-positive	103 (55.7%)	6 (11.8%)
Anti-CCP (+)	74 (40.0%)	0 (0%)
Anti-CCP (-)	29 (15.7%)	6 (11.8%)
RF (+)	67 (36.2%)	0 (0%)
RF (-)	36 (19.5%)	6 (11.8%)
MVP-negative	82 (44.3%)	45 (88.2%)
Anti-CCP (+)	38 (20.5%)	1 (1.9%)
Anti-CCP (-)	44 (23.8%)	44 (86.3%)
RF (+)	39 (21.1%)	2 (3.9%)
RF (-)	43 (23.2%)	43 (84.3%)

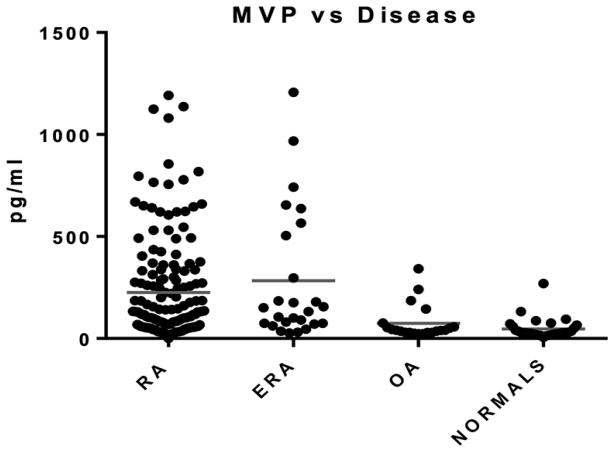

Fig. 2. Levels of MVP in patients with RA, early RA, OA and healthy individuals.

and t=3.85; p=0.0002, respectively). Similarly, MVP levels were higher in ERA and RA patients compared to OA patients (t=2.88; p=0.006 and t=2.71; p=0.008, respectively). There was no difference in MVP levels between OA patients and healthy controls (t=1.49; p=no significant [ns]) (Fig. 2).

Diagnostic characteristics of MVP

- MVP is highly associated with anti-CCP antibodies

The majority of RA patients were positive for MVP (55.7%), while 40% of these were positive for both MVP and anti-CCP. On the other hand, MVP positivity alone (without anti-CCP antibodies) was present in a small proportion of RA sera (15.7%) and anti-CCP-positivity alone (without MVP) was detected in 20.5% of RA. In the control group, 11.8% were positive for MVP, while 1.9% was positive for anti-CCP (Table II).

A significant association was found between MVP positivity and anti-CCP positivity (χ^2=33.9; p<0.00001). MVP levels were also significantly higher in anti-CCP-positive sera as compared to anti-CCP-negative sera (t= 7.59; p<0.0001). Finally, a modest but significant correlation was found between the anti-CCP and MVP levels using Pearson's correlation analysis (r= 0.197; p=0.0022) (Fig. 3).

- MVP is highly associated with the presence of RF

36.2% of the RA patients were positive for both MVP and RF. MVP-positivity with negative RF was present in 19.5% of cases, while RF alone was found in 21.1% of cases. 11.8% of the controls were found to be positive for MVP; 3.9% were found to be positive for RF and 0% was found to be positive for both MVP and RF (Table II).

A significant association between MVP and RF-positivity was detected (χ^2=20.1; p<0.00001). Moreover, the levels of MVP were significantly higher in RF-positive sera compared to RF-negative sera (t= 6.66; p<0.0001). Finally, Pearson's analysis demonstrated a statistical correlation between MVP and RF (r=0.420; p<0.0001) (Fig. 3).

- Lack of strong association between MVP and CRP in RA and ERA

A marginally significant association between MVP-positivity and CRP-positivity was observed (χ^2=6.5; p=0.011). However, no significant correlation was found between the anti-CRP and MVP levels using Pearson's analysis (r= 0.071; p=ns). Also, no difference was observed between the mean levels of CRP in MVP-positive and MVP-negative sera (t=0.03; p=ns).

Diagnostic value of serological parameters for RA

The diagnostic performance of the individual parameters (and their combinations) for RA and ERA is shown in Table III. MVP showed similar sensitivity (56%) and less specificity (88%) than anti-CCP and RF. However, the combined presence of MVP with either one of anti-CCP or RF or both increased the sensitivity, negative predictive value (NPV) and accuracy for RA compared to either MVP or RF or anti-CCP alone (Table IIIA). The presence of RF or anti-CCP antibodies (without considering the MVP) showed a sensitivity of 64%,

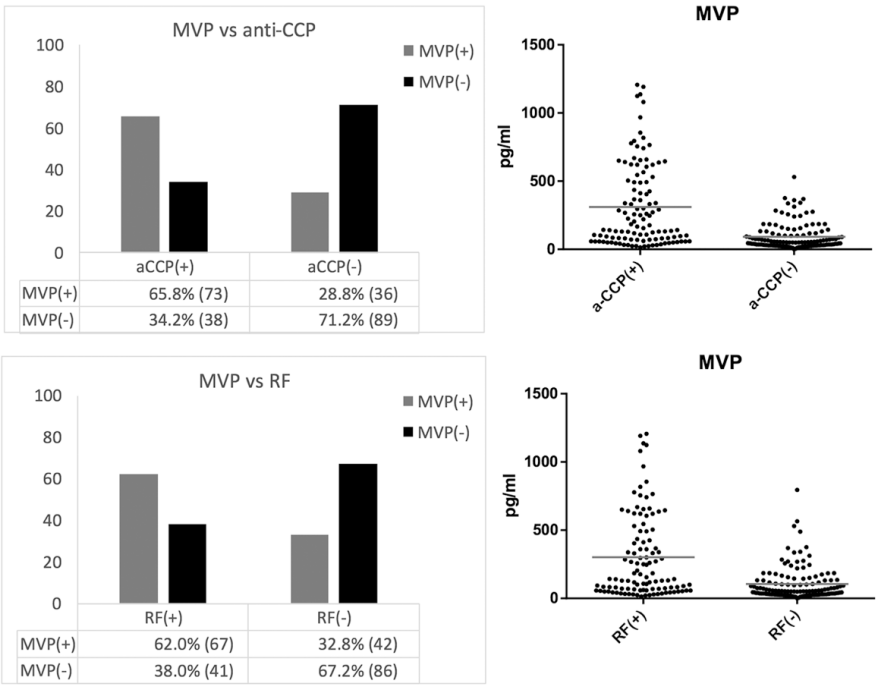

Fig. 3. MVP-positivity compared to anti-CCP or RF positivity, the percentage is presented using bars (the number of samples is enclosed in parenthesis) (left). The levels of MVP in anti-CCP or RF positive and negative samples (right).

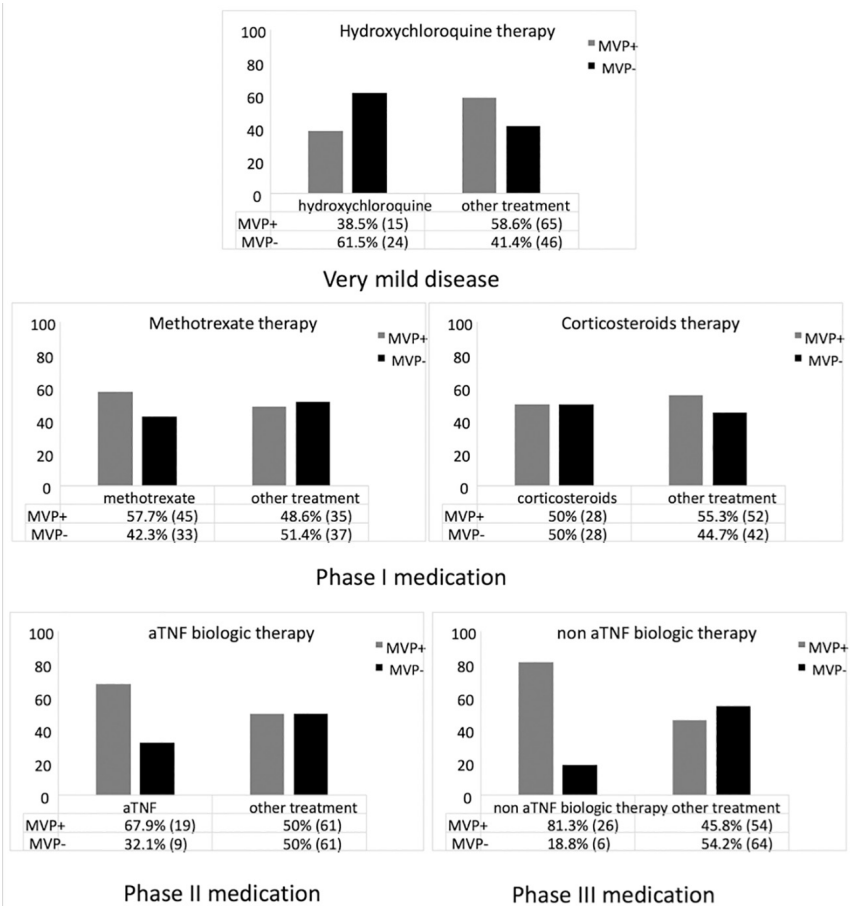

Fig. 4. MVP positivity compared to different medication schedules (hydroxychloroquine, methotrexate, corticosteroids, anti-TNF biologics, non anti-TNF biologics the percentage is presented using bars and the number of samples is enclosed in parenthesis.

specificity of 94% and an accuracy of 71%. Whereas, the addition of MVP improved the diagnostic performance, leading to a sensitivity of 78%, NPV 51% and an accuracy of 79%. On the other hand, CRP, which is a non-specific biomarker of inflammation, had a diagnostic accuracy of 60% for RA.

In the case of ERA, sensitivity of MVP (62%) was significantly higher than those of anti-CCP antibodies (42%) and RF (42%), but the specificity was slightly lower (88% *vs*. 98% and 96%, for anti-CCP and RF respectively). The combined presence of MVP with one of the anti-CCP or RF, or even both of them, significantly increased the sensitivity, NPV and accuracy for ERA compared to either MVP or RF or anti-CCP alone (Table IIIB). The presence of one of RF or anti-CCP antibodies (without considering the MVP) showed a sensitivity of only 46%, a specificity of 94% and an accuracy of 78%, whereas, the addition of MVP improved the diagnostic performance leading to a sensitivity of 77%, NPV 86% and an accuracy of 81%.

To analyse the diagnostic accuracy of serological parameters regarding ERA and RA, a ROC analysis was performed. The optimal cut-off value for MVP that best distinguished RA/ERA from non-RA/ERA was determined at the maximum value of Youden's index, which was estimated as (sensitivity + specificity - 1). Based on these results, a cut-off MVP concentration of 450 pg/mL yielded the highest Youden's index value for diagnosis of RA/ERA. In the case of established RA, the presence of anti-CCP had the highest AUC (0.909; 95% confidence interval (CI): 0.870–0.947) followed by RF (0.877; 95% CI: 0.827–0.928) and MVP (0.837; 95% CI: 0.777–0.896) (Fig. 5A). In the case of ERA, the presence of anti-CCP had the highest AUC (0.916; 95% confidence interval (CI): 0.848–0.984) followed by MVP (0.879; 95% CI, 0.798–0.961) and RF (0.876; 95% CI: 0.800–0.953) (Fig. 5B).

Clinical correlations of MVP

The clinical characteristics of MVP-positive/MVP-negative and anti-CCP-positive/anti-CCP-negative RA patients are shown in Table IV. A marginal statistically significant difference (t=2.02;

Table III. Test characteristics MVP, RF, and anti-CCP antibodies in RA and ERA.

A. Diagnostic value of MVP, RF and anti-CCP for rheumatoid arthritis

	Sensitivity	Specificity	PPV	NPV	Accuracy
MVP	0.56	0.88	0.94	0.35	0.63
anti-CCP	0.61	0.98	0.99	0.41	0.69
RF	0.57	0.96	0.98	0.38	0.66
RF OR anti-CCP	0.64	0.94	0.98	0.42	0.71
MVP OR anti-CCP	0.76	0.86	0.95	0.50	0.78
MVP OR RF	0.78	0.84	0.95	0.50	0.78
MVP OR anti-CCP OR RF	0.78	0.82	0.94	0.51	0.79
MVP OR anti-MVP-pep2	0.74	0.76	0.92	0.44	0.75
MVP OR anti-MVP-pep2 OR anti-CCP OR RF	0.88	0.69	0.91	0.61	0.84

B. Diagnostic value of MVP, RF and anti-CCP for early rheumatoid arthritis

	Sensitivity	Specificity	PPV	NPV	Accuracy
MVP	0.62	0.88	0.73	0.82	0.79
anti-CCP	0.42	0.98	0.91	0.77	0.79
RF	0.42	0.96	0.85	0.77	0.78
RF OR anti-CCP	0.46	0.94	0.80	0.77	0.78
MVP OR anti-CCP	0.73	0.88	0.76	0.87	0.83
MVP OR RF	0.77	0.84	0.71	0.88	0.82
MVP OR anti-CCP OR RF	0.77	0.82	0.69	0.86	0.81
MVP OR anti-MVP-pep2	0.77	0.76	0.63	0.86	0.76
MVP OR anti-MVP-pep2 OR anti-CCP OR RF	0.88	0.69	0.61	0.92	0.76

Includes positive predictive value (PPV), negative predictive value (NPV).

p=0.050) was observed between MVP-positive and MVP-negative patients, using the "Larsen score". The statistical difference in the "Larsen score" (t=0 years) was slightly stronger regarding the anti-CCP antibody levels between these groups (t=2.6; p=0.011). Additionally, the levels of anti-CCP-antibodies were found to be moderately correlated using the HAQ-DI score (t=2.2; p=0.034). No other clinical parameters or disease activity indices were significantly correlated with the levels of either MVP or anti-CCP.

MVP with regard to treatment

Therapy with hydroxychloroquine was negatively correlated with MVP positivity (χ^2=4.68; p=0.030), while lower MVP levels were detected in patients receiving hydroxychloroquine (t=2.14; p=0.03). No significant correlation was found between MVP-positivity and a treatment with methotrexate or corticosteroids DMARDs. TNF-inhibitor biological therapy was not significantly correlated with MVP (χ^2=2.92; p=ns), although there was a trend to have higher MVP levels than other therapies. A significant positive correlation was observed between MVP-positivity and non anti-TNF biologics (χ^2=12.74; p=0.00036). Patients treated with non-TNF inhibitor biologics, in contrast to those treated with hydroxychloroquine, showed higher MVP-levels compared to patients receiving another therapy (Fig. 4).

According to the EULAR treatment algorithm (12) MVP levels were found to correlate well with the treatment given to each therapeutic phase (r=0.9995; p<0.0005, Fig. 6). Since an upgraded treatment plan (next medication phase) is required in the case of unmet therapeutic goals, one could suggest that MVP-levels are proportional to the number of therapeutic failures and subsequent medication changes. In other words, a patient with high MVP con-

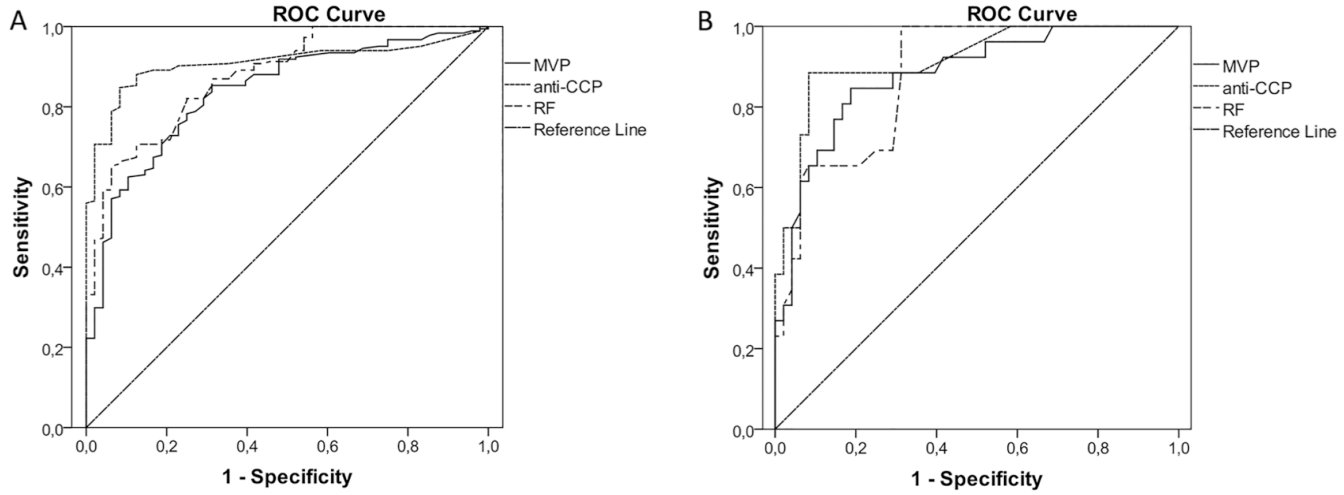

Fig. 5. ROC analysis in RA and ERA patients for anti-CCP, RF and MVP

A: ROC analysis in RA patients for anti-CCP AUC (0.909; 95% confidence interval [CI], 0.880–0.953; p<0.001), RF (0.877; 95% CI, 0. 827–0.928; p<0.001.) and MVP level (0.837; 95% CI, 0.777–0.896; p<0.001) in RA.

B: ROC analysis in ERA patients for anti-CCP AUC (0.916; 95% confidence interval [CI], 0.848–0.984; p<0.001), RF (0.876; 95% CI, 0.800–0.953; p<0.001) and MVP (0.879; 95% CI, 0.798–0.961; p<0.001).

Table IV. Clinical characteristics of established RA patients (with no ERA) in relation to MVP and anti-CCP antibodies.

Characteristic	MVP positive	MVP negative	*p*	Anti- CCP positive	Anti- CCP negative	*p*
t=0 years						
HAQ-DI	1.2 ± 0.8	1.2 ± 0.9	ns	1.3 ± 0.9	0.9 ± 0.8	0.034
Larsen score	51.6 ± 28.5	39.4 ± 22.9	0.050	51.4 ± 27.2	35.0 ± 23.6	0.011
TJC	8.9 ± 7.1	8.8 ± 7.9	ns	8.9 ± 7.9	8.7 ± 7.0	ns
SJC	5.1 ± 5.9	5.2 ± 6.6	ns	5.8 ± 6.6	4.1 ± 5.7	ns
VAS	37.8 ± 20.7	41.1 ± 21.8	ns	40.2 ± 22.4	39.1 ± 20	ns
DAS28	4.8 ± 1.2	4.6 ± 1.7	ns	4.8 ± 1.6	4.5 ± 1.3	ns
t=2 years						
HAQ-DI	0.9 ± 0.8	0.7 ± 0.8	ns	0.9 ± 0.9	0.7 ± 0.8	ns
Larsen score	50.1 ± 32.4	38.5 ± 27.5	ns	48.9 ± 32.0	36.5 ± 26.2	ns
TJC	6.0 ± 7.5	9.5 ± 10.5	ns	6.1 ± 7.8	11.2 ± 11.1	ns
SJC	4.6 ± 4.6	2.8 ± 4.2	ns	3.7 ± 4.2	3.3 ± 4.9	ns
VAS	29.7 ± 23.7	26.5 ± 20.3	ns	28.1 ± 23.2	27.8 ± 19.5	ns
DAS28	4.1 ± 1.5	3.8 ± 1.6	ns	3.9 ± 1.5	4 ± 1.6	ns

VAS: Visual analogue scale; TJC: tender joint count; SJC: swollen joint count; DAS28: Disease Activity Score using 28 joint count; HAQ-DI: Health Assessment Questionnaire Disability Index; ± represents ± Standard Deviation.

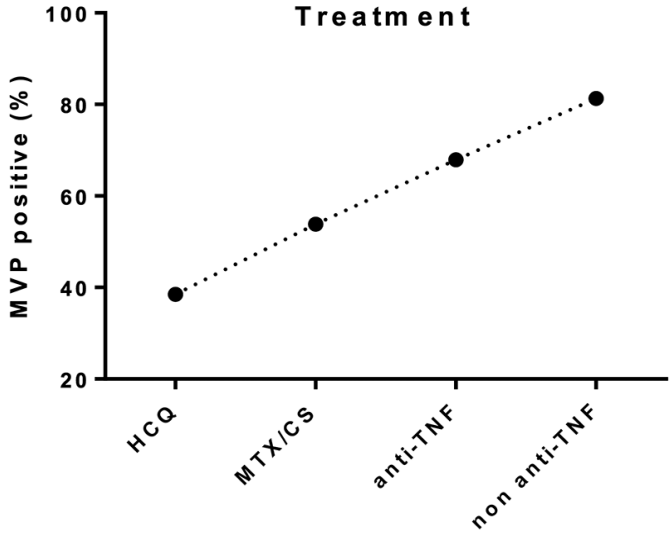

Fig. 6. Percentage of MVP-positivity in different medication phases. Mild therapy: hydroxychloroquine (HCQ), Phase I: methotrexate (MTX), corticosteroids (corticosteroids), Phase II: anti-TNF biologics, Phase III: non anti-TNF biologics.

centration is more likely to be a non-responder to the treatment (Fig. 6).

Prevalence of antibodies against MVP peptides in rheumatoid arthritis

The ability of MVP to act as an autoantigen was examined using 2 synthetic peptides representing two predicted epitopes, highly exposed in MVP structure (Fig. 1B). A significant correlation was found between anti-MVP-pep2-positivity and RA (χ^2=14.13; p=0.0002), whereas no significant correlation was found between anti-MVP-pep1-positivity and RA (χ^2=0.12; p=0.75) (Fig. 7). Furthermore, anti-MVP-pep2 antibodies were detected in higher levels in patients with RA compared to normal controls (t=2.73; p=0.007). On the other hand, no difference in anti-MVP-pep1 antibody levels was observed between RA and OA or healthy controls.

Moreover, there was a significant association between anti-MVP-pep2 and RF-positivity (χ^2=8.73; p=0.003), as well as between anti-MVP-pep2-positivity and the levels of their antigenic target MVP (χ^2=4.61; p=0.03). No significant association was found between anti-MVP-pep2-positivity and anti-CCP or CRP positivity (χ^2=3.52; p=0.04, χ^2=0.04; p=0.84, respectively). Furthermore, no significant correlation was found between the levels of anti-MVP-pep2 antibodies and anti-CCP, RF or CRP levels (r=0.015; p=ns, r=0,001; p=ns, r=0,04; p=ns, respectively). MVP-pep1 was not associated with any of RF, anti-CCP, MVP or CRP parameters.

Interestingly, the combined and exclusive use of anti-MVP-pep2 and MVP as diagnostic marker for the detection of RA led to an accuracy of 75% with a sensitivity of 74%, a specificity of 76% and a positive predictive value of 92% (Table IIIA). Moreover, the addition of one of the anti-CCP or RF, or even both of them, to MVP OR anti-MVP-pep2 significantly increased the sensitivity, NPV and accuracy, leading to an accuracy of 84% in the case of RA.

Discussion

In this study, we evaluated the diagnostic accuracy of MVP for RA and the usefulness of this test compared to others (*i.e.* anti-CCP antibodies and RF) used in clinical practice.

Nowadays, serological diagnosis of RA is based on the detection of anti-CCP and RF (13). Anti-CCP antibodies are the most specific biomarker for the disease (14). However, no single biomarker has the potential to achieve early diagnosis of RA to date. Early diagnosis and early institution of effective treatment can prevent the progressive damage of joints, improve the functional status, likely to achieve disease remission, and improve quality of life (15).

In the present study, it was observed that the levels of MVP were high in patients with RA and ERA compared to OA and healthy individuals, suggesting that MVP could be a useful biomarker for RA. Indeed, MVP showed the potential to discriminate RA/ERA from non-RA patients with high accuracy. We also compared the diagnostic value of MVP for RA and ERA *versus* RF and anti-CCP antibodies. In our cohort, MVP showed similar accuracy for the diagnosis of RA to anti-CCP and RF. In the case of ERA, MVP demonstrated higher sensitivity than RF and anti-CCP antibodies, but equal accuracy to them. In addition, the AUC of MVP was slightly higher than the AUC of RF for ERA patients. Remarkably, the combination of "MVP OR (RF OR anti-CCP)" showed improved accuracy compared to RF or anti-CCP alone, or even the combination of "RF OR anti-

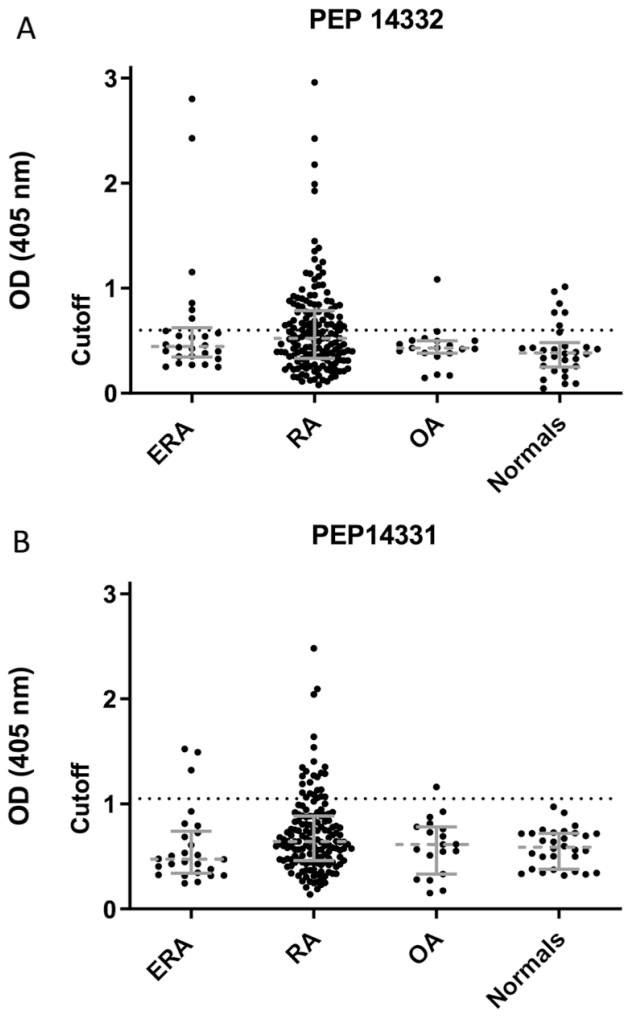

Fig. 7. Levels of anti-MVP-pep2 and anti-MVP-pep1 antibodies in patients with RA, early RA, OA and healthy individuals.
A: Levels of autoantibodies against MVP-pep2.
B: Levels of autoantibodies against MVP-pep1. Median with interquartile range is depicted for each group of sera in grey.

CCP" for either RA or ERA patients. Thus, MVP has a significant potential to serve as an additional biomarker over and above the established ones.

The value of MVP in the prediction of the outcome of RA, the clinical signs of the disease activity, and the severity of joint damage was also investigated. In this regard, we examined possibility of a correlation between MVP and various parameters of disease activity in RA. In the literature, anti-CCP antibody levels correlated with Larsen score and HAQ-DI score but not with VAS, DAS28, tender joint count, swollen joint count (16-19). In our cohort, MVP showed a similar association to anti-CCP with the Larsen score and a borderline significant trend in association with HAQ-DI. Taken together, MVP, like anti-CCP, slightly correlated with the radiographic joint damage and is not a very useful marker for the assessment of disease severity.

Next, we examined if MVP could be correlated with the type of treatment administered to RA patients or if it possessed a prognostic value regarding the response to treatment. According to the EULAR treatment for RA algorithm (12) and 2015 ACR management recommendations (20), there is a hierarchy concerning the treatment options for RA. Hydroxychloroquine (HCQ) is used in very mild or palindromic disease, while methotrexate (MTX), alone or in combination with corticosteroids (CS), is used as a first management approach (phase I). Failure to meet treatment objectives and achieve improvement leads to the addition of (or switch to) a biologic agent, usually an anti-TNF agent (phase II). Lack of efficacy, treatment failure and/or toxicity in phase II requires the replacement of the first biologic agent with another agent directed to a different target (*i.e.* rituximab - CD20, abatacept – CD28 and tocilizumab - IL-6R) or another TNF-inhibitor (phase III). MVP-positivity correlated well with the therapeutic phase, *i.e.* MVP-positivity is less frequently observed in patients treated with HCQ < patients treated with MTX/CS < patients treated with anti-TNF < patients treated with non-anti-TNF. Since failure to meet the objectives of treatment requires the administration of next phase medication, one could hypothesise that MVP-positivity is proportional to the number of therapeutic failures and subsequent medication changes. In other words, a patient with high MVP-concentration (MVP-positive) is more likely to have a treatment resistant disease. This hypothesis is of particular interest since the MVP protein is also known as "Lung resistance-related protein (LRP)" and is related to patients' resistance to chemotherapy in certain types of cancer. High LRP/MVP expression has a predictive value for multidrug resistance and poor outcome in patients with cancer (21). Although, our study was not designed prospectively to safely assess whether MVP correlates with therapeutic failure in RA, the directly proportional relation between MVP-positivity and the phase of therapy strongly implies that it might be the case.

MVP involvement in the molecular pathways of RA has started to emerged. Recently, it was found that MVP suppresses the TLR/IL-1R associated IKK - NF-κB signal pathway. MVP (or probably the whole vault complex) interacts with TRAF6 and inhibits the formation of the TRAF6-IRAK complex and TRAF6 polyubiquitinilation (Fig. 8). Given that the recruitment of TRAF6 to IRAK promotes its oligomerisation and the activation of NF-κB via IKK-NF-κB cascades (22), MVP has a pivotal role in the inhibition of this pathway that triggers the production of pro-inflammatory cytokines and onset of inflammation. Moreover, TRAF6 that is not in complex with IRAK1 has the potential to inhibit another pathway that is crucial for the induction of chronic inflammation in RA, the TNF-α pathway. Previous reports suggested that TRAF6 negatively regulates TNF-α induced NF-κB activation upstream of IKK (23). TLR/IL-1R and TNF-α

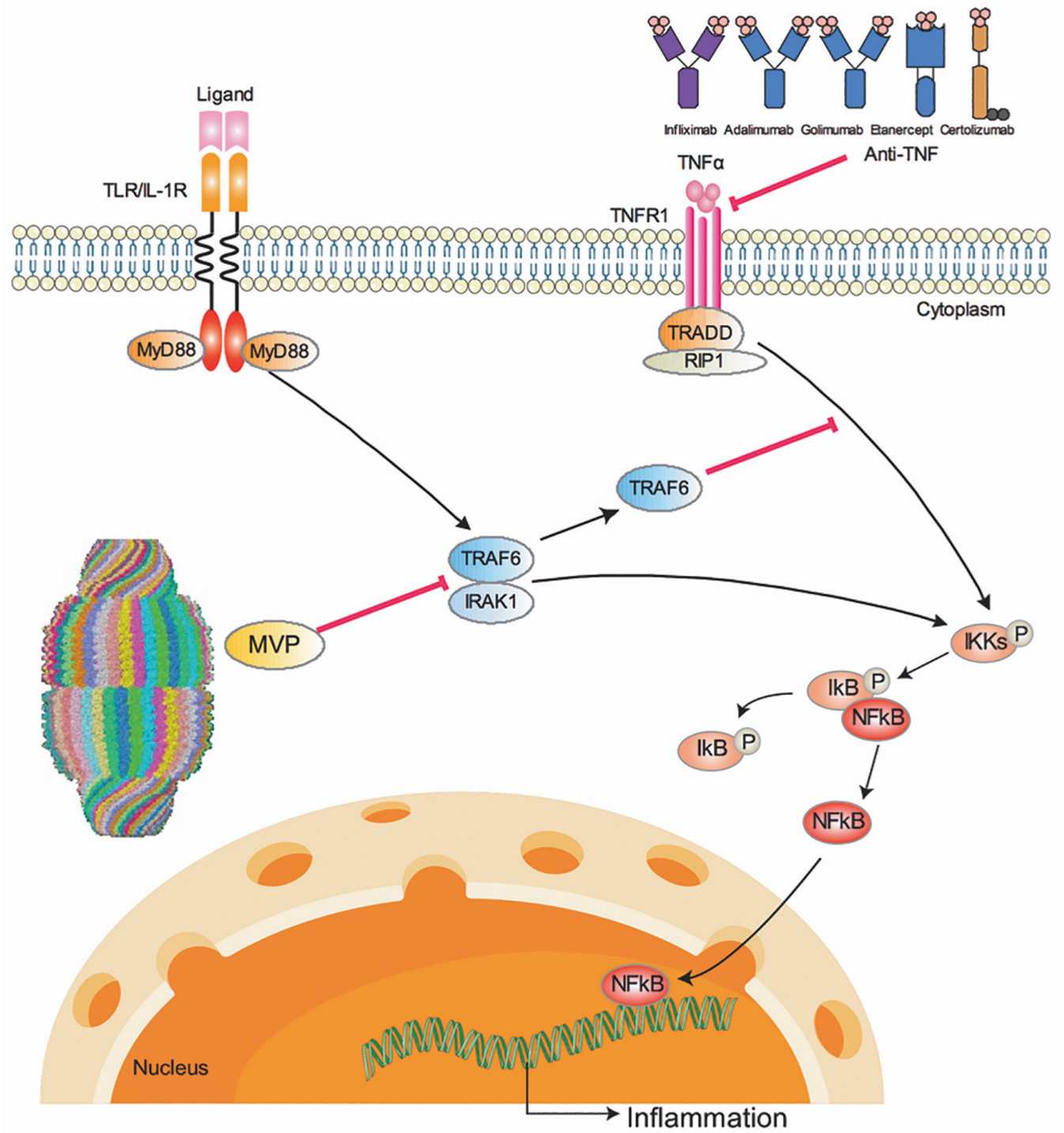

Fig. 8. Hypothetical model for MVP function in signalling pathways that control NF-kB activation to the process of inflammation in RA. Median with interquartile range is depicted for each group of sera in grey.

pathways overlap when considering the points of activated kinases and transcription factors, as well as the induced expression of inflammatory cytokines. TRAF6 is a molecule that can be involved in both signaling pathways (Fig. 8) and it is also known that TRAF6 is overexpressed in RA (24, 25). Taken together, increased MVP levels could be produced in the human body in an attempt to reduce inflammation or limit it to a "low grade and chronic" state by inhibiting the NF-κB signal transduction. Maybe these levels are elevated in patients who do not respond to therapy, since they usually have higher levels of inflammation than the responders.

MVP itself might also be a target of autoantibodies. According to a study of Monach *et al*., MVP is co-purified with IgG extracted from immune complexes deposited in joint tissues in RA (26). Also, antibodies against vaults (27) and against MVP were detected in systemic lupus erythematosus (SLE) (27-29). Taking into account the full structure of the vault particle, two epitopes on

MVP (MVP-pep1 and MVP-pep2) were designed to cover exposed and predicted antigenic regions on the 3D structure of the molecule. RA patients showed significantly higher levels of anti-MVP-pep2 autoantibodies, compared to healthy controls. Moreover, anti-MVP-pep2 autoantibody-positivity correlated with the MVP-positivity, leading to the conclusion that patients who produce anti-MVPpep2 antibodies manifest high levels of their antigenic target (MVP). It is not a far-fetched concept that MVP and anti-MVP-autoantibodies may be form complexes in RA and deposited in joints.

In conclusion, we identified MVP as a novel biomarker that can be used together with anti-CCP and RF for RA-diagnosis. Moreover, MVP might be a prognostic marker for resistance to treatment.

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