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# Peptides Bearing Multiple Post-Translational Modifications as Antigenic Targets for Biomarkers towards Personalized Rheumatology

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## Introduction

Rheumatoid arthritis (RA) is an autoimmune rheumatic disease characterized by the presence of autoantibodies resulting from an immune response triggered by proteins that have undergone post-translational modifications (PTMs) [1]. Citrullination (deimination of arginine), is the best-studied PTM in rheumatology, and for a long time, anti-citrullinated protein/peptide antibodies (ACPAs) have been used for RA diagnosis and considered the most relevant serological markers. More recently, other PTMs have been described in the context of RA including homocitrullination (carbamylation) and acetylation, which take place in lysine (Figure 1). Different autoantibodies against these novel modifications (antibodies against modified proteins/peptides, AMPAs) have been linked with interstitial lung disease (ILD), an extra-articular manifestation of RA that entails a high mortality [2,3].

## Results and Discussion

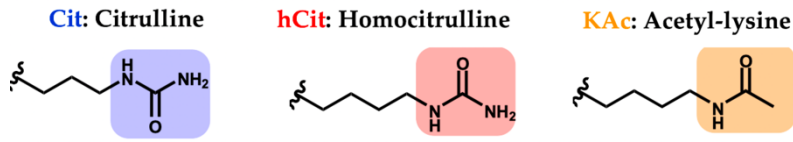


*Fig. 1. Chemical structures of relevant PTMs in RA. Citrulline results of the conversion from arginine while homocitrulline and acetyl-lysine come from the conversion of lysine residues.*

Previous studies in our group defined a chimeric citrullinated peptide containing α-fibrin and filaggrin domains (CFFCP1, Figure 2) that yielded better results in terms of sensitivity/specificity balance and identified RA patients with poor radiographic outcomes as an antigen compared to the gold standard commercial test [4]. Based on the primary structure of this peptide, a panel of several peptide antigens that resulted from the substitution of lysine for homocitrulline and/or acetyl-lysine were designed (Figure 2), synthesized by solid-phase and tested in a RA population (n=37 RA-ILD vs n=141 RA non-ILD) to study their role as biomarkers linked to the presence of ILD.

The analysis of fine specificities with these novel chimeric peptide antigens demonstrated that IgG was the predominant isotype in the main RA population. IgG and IgA isotypes were more frequent when the antigen was doubly modified (citrullinated/homocitrullinated) compared to anti-ACPA specificities. In addition, all anti-AMPA fine specificities were more frequent in the RA-ILD group. Regarding the IgA isotype, the peptide containing two or three PTMs was able to detect a percentage close to 20% of RA-ILD sera that were negative when analyzed with the peptide bearing a single PTM. Particularly, the mean titers of autoantibodies against the peptide antigen bearing three PTMs, citrullinated/homocitrullinated/acetylated chimeric peptide (CFFCHAP) were statistically significant for IgA and IgM isotypes, but also for IgG ($p < 0.05$) (Figure 3), a finding not observed with the ones against doubly PTM-modified antigens.

| Chimeric peptide | α-fibrin(617-631)-S$^{306}$,S$^{319}$ cyclo [Cys$^{306,319}$] filaggrin(304-324) |
|---|---|
| CFFCP1 | HSTKRGHAKSRPV**Cit**G-HQC**H**QEST**Cit**GRSRGRC**G**RSGS |
| CFFCHP1 | HSTKRGHA**hCit**SRPV**Cit**G-HQC**H**QEST**Cit**GRSRGRC**G**RSGS |
| CFFCHP2 | HST**hCit**RGHAKSRPV**Cit**G-HQC**H**QEST**Cit**GRSRGRC**G**RSGS |
| CFFCHP3 | HST**hCit**RGHA**hCit**SRPV**Cit**G-HQC**H**QEST**Cit**GRSRGRC**G**RSGS |
| CFFCHAP | HST**KAc**RGHA**hCit**SRPV**Cit**G-HQC**H**QEST**Cit**GRSRGRC**G**RSGS |

*Fig. 2. Primary structure of synthetic cyclic fibrin-filaggrin chimeric peptides bearing citrulline, homocitrulline and acetyl-lysine at specific positions of the α-fibrin (617-631) and cyclic filaggrin (304-324) domains.*

When analyzing patients according to the presence of erosive disease assessed by radiographs of hands and feet (Larsen score), a higher prevalence of autoantibodies was found in patients with more severe joint damage (Larsen ≥ 18) in comparison to those with mild joint destruction (Larsen < 18). The differences were statistically significant in almost all specificities, and especially for IgA and IgM isotypes. Interestingly, only the frequency of antibodies of the IgG isotype against the triple PTM peptide (anti-CFFCHAP-IgG-positive) was higher in patients with severe joint damage, with statistical significance.

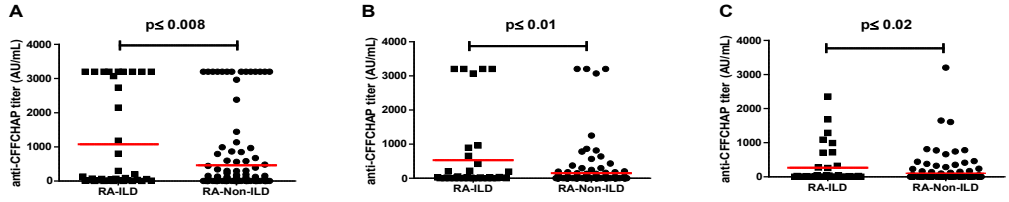


*Fig. 3. Comparison of anti-CFFCHAP titers (AU/mL) for RA-ILD vs RA-non-ILD patients. A) IgG; B) IgA and C) IgM isotypes. Horizontal lines depict mean titers. Statistical significances were established as two-tailed p-values < 0.05 after analyzing the continuous variables using the Mann–Whitney U test.*

For the first time, we described a novel peptide-based antigen bearing citrulline, homocitrulline and acetyl-lysine into the same peptide sequence to consistently detect severe phenotypes in CFFCHAP positive RA patients. We demonstrated an association between the presence and levels of AMPA titers and severe manifestations of RA such as joint destruction and ILD [5].

## Acknowledgments

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