Autoimmunity

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/iaut20

# Alteration of telomere length and mtDNA copy number in interstitial lung disease associated with rheumatoid arthritis

Giada De Benedittis, Andrea Latini, Chiara Morgante, Chiara Bonini, Eneida Cela, Barbara Kroegler, Alessandra Luciano, Marcello Chiocchi, Francesco Cavalli, Josuel Ora, Paola Rogliani, Giuseppe Novelli, Cinzia Ciccacci, Maria Sole Chimenti, Paola Conigliaro & Paola Borgiani

**To cite this article:** Giada De Benedittis, Andrea Latini, Chiara Morgante, Chiara Bonini, Eneida Cela, Barbara Kroegler, Alessandra Luciano, Marcello Chiocchi, Francesco Cavalli, Josuel Ora, Paola Rogliani, Giuseppe Novelli, Cinzia Ciccacci, Maria Sole Chimenti, Paola Conigliaro & Paola Borgiani (2025) Alteration of telomere length and mtDNA copy number in interstitial lung disease associated with rheumatoid arthritis, Autoimmunity, 58:1, 2473741, DOI: 10.1080/08916934.2025.2473741

**To link to this article:** https://doi.org/10.1080/08916934.2025.2473741



Published online: 04 Mar 2025.

AUTOIMMUNITY
2025, VOL. 58, NO. 1, 2473741
https://doi.org/10.1080/08916934.2025.2473741

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

# Alteration of telomere length and mtDNA copy number in interstitial lung disease associated with rheumatoid arthritis

Giada De Benedittis[a], Andrea Latini[b], Chiara Morgante[a], Chiara Bonini[c], Eneida Cela[c], Barbara Kroegler[c], Alessandra Luciano[d], Marcello Chiocchi[d], Francesco Cavalli[e], Josuel Ora[e], Paola Rogliani[e], Giuseppe Novelli[a,f], Cinzia Ciccacci[b], Maria Sole Chimenti[c], Paola Conigliaro[c#] and Paola Borgiani[a#]

[a]Department of Biomedicine and Prevention, Section of Genetics, University of Rome "Tor Vergata", Rome, Italy; [b]UniCamillus–Saint Camillus International University of Health Sciences, Rome, Italy; [c]Rheumatology, Allergology and Clinical Immunology, Department of System Medicine, University of Rome "Tor Vergata", Rome, Italy; [d]Department of Diagnostic Imaging and Interventional Radiology, University of Rome Tor Vergata, Rome, Italy; [e]Division of Respiratory Medicine, University Hospital Policlinico Tor Vergata, Rome, Italy; [f]Giovanni Lorenzini Medical Foundation, Milan, Italy

**ABSTRACT**

Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA). The inflammatory response in lung disease is characterized by severe oxidative stress, which enhances cellular senescence. Telomeric shortening and mitochondria dysregulation represent two hallmarks of cellular senescence. The maintenance of telomere length (TL) and mitochondrial DNA (mtDNA) copy number is preserved by many proteins, such as TERF1 and TFAM, respectively. Our aim was to evaluate the TL, the mtDNA copy number and the expression of two regulator gene factors in RA patients with (RA-ILD) and without lung involvement (RA-NILD). Eighty-five RA patients and 21 healthy subjects were enrolled. Relative TL, mtDNA copy number, and expression analysis of *TERF1* and *TFAM* genes were measured using qPCR assay. All RA patients present a statistically significant telomere shortening; in particular, RA-ILD patients show shorter TL compared to both controls and RA-NILD. Patients with Usual Interstitial Pneumonia pattern show a more evident shortening of TL. Lastly, both RA-ILD and RA-NILD patients present a significant decrease in mtDNA copy number compared to controls. The analysis of regulatory genes showed an increase in *TERF1* expression in RA patients compared to controls, also after stratification in the two subgroups, and a decrease in *TFAM* expression in RA patients compared to controls. These results show that the alteration of TL and mtDNA copy number in RA patients is more evident in the presence of ILD. The hypothesis is that, in these patients, oxidative stress could accelerate the shortening of telomeres and the decrease of mtDNA copy number.

**ARTICLE HISTORY**
Received 6 December 2024
Revised 4 February 2025
Accepted 14 February 2025

**KEYWORDS**
Rheumatoid arthritis; interstitial lung disease; telomere length; mtDNA; gene expression

## 1. Introduction

Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA) that strongly contributes to increased morbidity and mortality [1]. ILD occurs in the presence of an abnormal healing response due to an injury of the lungs; consequently, the tissue around the alveoli becomes scarred and thickened, leading over time to a fibrotic tissue [2]. The most common histopathological patterns recognized are usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), organizing pneumonia (OP), and acute lung injury (ALI) [3]; among these, the UIP pattern represents the most prevalent and severe one [4]. Furthermore, some patients cannot be included in these patterns and are consequently labeled as unclassifiable (UN) ILD [5]. The development of ILD in RA patients is the result of a complex interplay among genetic, environmental, and autoimmune factors contributing to the aberrant inflammatory response in the pulmonary parenchyma and alveolar wall [6]. ILD, in its prototypical form, that is idiopathic pulmonary fibrosis, can be viewed in part as an aberration of the normal aging process of the lung. Indeed, to date, the recognized risk factors involved in the development of ILD are aging, smoking, male gender, positivity for anti-citrullinated protein antibodies (ACPA) and high disease activity [7,8]. However, cellular senescence has not been deeply studied in RA-ILD as a pathogenetic mechanism leading to tissue damage.

Since oxidative stress (OS) plays an important role in the inflammatory responses of several lung diseases [9,10], it could be included to the ILD risk factors; in fact, it represents one of the main causes of DNA damage leading to cellular senescence. Telomeres seem particularly sensitive to OS

**CONTACT** Paola Borgiani borgiani@med.uniroma2.it Department of Biomedicine and Prevention, Section of Genetics, University of Rome "Tor Vergata", Rome, Italy.
[#]These authors have contributed equally to this work.

damage, that can accelerate their shortening [11]. For this reason, telomere shortening is frequently used as a biomarker of cellular senescence. Telomeres are specific DNA regions of repetitive "TTAGGG" nucleotide sequences that protect chromosome ends and preserve genetic information. They progressively shorten during each cell division as a part of the cell aging process, leading lastly to cell cycle arrest and apoptosis when reaching a critical point. The integrity of telomeres is preserved by several proteins that are part of the shelterin complex; the telomeric repeat-binding factor 1 protein (TERF1) is part of this complex and is essential for maintaining telomere length (TL), acting as a telomerase inhibitor. Shortened telomeres have been described in RA and idiopathic pulmonary fibrosis [12,13]. However, few data are present in the literature regarding TL in RA-ILD [14,15], while no data are available regarding TERF1 in RA.

Another recognized hallmark of cellular senescence is mitochondria dysfunction [16]; indeed, histones and DNA repair are absent in mitochondrial DNA (mtDNA), which makes it more exposed to OS than nuclear DNA [17]. As a result, mtDNA accumulates mutations and mtDNA copy number decreases over time, contributing to mitochondria dysfunctions [18]. The transcription of mtDNA during mitochondrial biogenesis is carried out by the mitochondrial transcription factor A (TFAM), which is localized in mitochondria but encoded by nuclear genes [19]. This factor also seems to protect and prevent damage from reactive oxygen species (ROS), in fact, it has been reported that *TFAM* overexpression accelerates the recovery of mtDNA levels following OS damage in rats' models [20]. A decreased in mtDNA copy number content have been yet described in RA [21]. However, no data regarding mtDNA copy number and TFAM in RA-ILD are available in the article.

Therefore, our aim was to investigate biomarkers of cellular senescence in a population of RA-ILD patients; in particular, we evaluated the TL and mtDNA copy number and the expression of their regulator gene factors (*TERF1* and *TFAM*) in RA patients with and without lung involvement.

## 2. Material and methods

### 2.1. Patients recruitment

We conducted a cross-sectional study on consecutive patients affected by RA-ILD ($n = 35$) and RA without ILD (RA-NILD, $n = 50$) referred to the Rheumatology Outpatient Clinic at the Department of Systems Medicine (University of Rome "Tor Vergata") between 1st January 2022 and 31st December 2023. Twenty-one age- and sex-matched healthy subjects (CTRLs) were also enrolled as controls. Blood sample from all subjects was obtained by standard antecubital venepuncture and stored at −20 °C until usage.

RA patients were classified according to the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria [22]. Demographic and clinical data were collected, including the presence of rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA) in the disease history, the onset date of RA and ILD, disease activity assessed by disease activity score on 28 joints (DAS28), ESR (mm/h), C-reactive protein (CRP, mg/dl), the presence of comorbidities, past and current pharmacological history (conventional synthetic (cs) disease modifying antirheumatic drugs (DMARDs), biologic (b) DMARDs, and targeted synthetic (ts) DMARDs, and smoking status (current, former, or never).

RA-ILD status was determined through standardized medical record. Specifically, RA-ILD classification required a provider diagnosis of ILD based on chest tomography (CT) findings (e.g. honeycombing, reticulation, traction bronchiectasis, groundglass opacities, centrilobular nodules, or cysts, among others). Patterns of RA-ILD were based on clinical reads of chest CTs performed by two blinded pneumologist and two radiologist and classified as UIP, nonspecific interstitial pneumonia (NSIP) or unclassified-ILD (UN-ILD).

All patients provided informed consent prior to enrollment and the study was approved by the local ethics committee of the Policlinico "Tor Vergata" in Rome (Approval No. RS162/21).

### 2.2. Telomere length and mtDNA copy number evaluation

Nuclear and mitochondrial DNA was extracted from PBMCs using a Qiagen blood DNA mini kit. The TL measurement was performed adapting the protocol described by Cawthon [23]. Relative TL was measured using quantitative PCR (qPCR) assay, which quantifies a ratio of telomeric repeat copy signal (TEL) and a reference single-copy gene signal (β-globin [HBB]). The Ct values were concurrently determined in each sample during the same qPCR run. The relative TL in leukocytes of each subject was calculated as T/S ratio ($=2^{-(Ct\ (TEL)-Ct\ (HBG))}$). Successively, RA patients were divided according to their TL (within or above the first quartile of the cohort).

The mtDNA copy number analysis was performed as described by Rooney [24]. Primers amplifying a nuclear DNA region (hemoglobin subunit beta [HGB]) and a mtDNA region (NADH dehydrogenase subunit 1, [ND1]) were available in the literature [25]. The Ct values for the nuclear HGB gene and mitochondrial ND1 gene were concurrently determined in each sample during the same qPCR run. The mitochondrial copy number in the leukocytes of each subject was calculated by the equation ($2 \times 2^{\ (Ct\ (HGB)-Ct\ (ND1))}$) [25].

All the reactions were performed in triplicate and using ABI 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, CA, USA) (SYBR Green Assay, Applied Biosystems).

### 2.3 mRNA isolation and expression analysis

Total RNA was extracted from PBMCs using TRIzol reagent (Ambion, CA, USA) protocol and, then, reverse transcribed it using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA). Expression analysis of *TERF1* and *TFAM* genes was performed by qPCR assay using ABI 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, CA, USA) (SYBR Green Assay,

Applied Biosystems). Each sample was analyzed in triplicate and, to standardize the results, each assay was run with an endogenous control (β-Actin). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

### 2.4. Statistical analysis

According with the Kolmogorov-Smirnov test, Mann–Whitney *U*-test and Kruskal–Wallis H-test, with Bonferroni correction, were used to compare TL, mtDNA copy number and gene expression levels among the different phenotypic groups. A multivariate logistic regression analysis was used to correct the *p*-value for sex, age, disease duration and CRP. Possible linear correlations were assessed by Pearson correlation analysis. A *p*-value ≤ 0.05 was considered significant in all tests. Data are shown as median and interquartile range. Statistical analysis was conducted using SPSS program ver. 19 (IBM Corp., Armonk, NY, USA), while all graphs were performed by GraphPad Prism 9 (GraphPad Software, USA).

## 3. Results

### 3.1. Patients clinical characteristics

Eighty-five RA patients were enrolled [(mean age 63.9 ± 12.3 years, median disease duration 9 years (interquartile range: 4–15)]: 50 RA-NILD and 35 RA-ILD. RA-ILD patients were older and with minor disease duration that RA-NILD patients. They exhibited higher CRP levels, positivity for RF and ACPA than RA-NILD patients (Table 1).

**Table 1.** Patients clinical characteristics.

| | RA (*n* = 85) | RA-NILD (*n* = 50) | RA-ILD (*n* = 35) | *p* (RA-NILD vs RA-ILD) |
|---|---|---|---|---|
| Female, *n* (%) | 68 (80.0) | 43 (86.0) | 25 (71.4) | ns |
| Age, years | 63.9 ± 12.3 | 60.4 ± 12.4 | 68.9 ± 10.5 | **0.002** |
| RA disease duration, years median (IQR) | 9 (4–15) | 12 (5–16) | 7 (4–10) | **0.04** |
| *Smokers* | | | | |
| Never, *n* (%) | 52 (61.2) | 33 (66.0) | 19 (54.3) | ns |
| Former, *n* (%) | 3 (3.5) | 3 (6.0) | 0 (0) | |
| Current, *n* (%) | 29 (34.1) | 13 (26.0) | 16 (45.7) | |
| RF, *n* (%) | 68 (80.0) | 34 (68.0) | 34 (97.1) | **0.001** |
| ACPA, *n* (%) | 61 (71.8) | 29 (58.0) | 32 (91.4) | **0.001** |
| DAS28, median (IQR) | 3.8 (2.4–4.6) | 3.6 (2.1–4.5) | 4.0 (3–4.7) | ns |
| CRP (mg/dl), median (IQR) | 0.6 (0.4–1.6) | 0.5 (0.3–1) | 1.4 (0.4–2.3) | **0.003** |
| csDMARDs, *n* (%) | 49 (57.7) | 27 (54.0) | 22 (62.9) | ns |
| Nintedanib, *n* (%) | 2 (2.3) | 0 (0) | 2 (5.7) | ns |
| bDMARDs, *n* (%) | 71 (84) | 45 (90) | 26 (75) | ns |
| ILD pattern | | | 15 (42.8) | |
| UIP, *n* (%) | | | 9 (25.7) | |
| NSIP, *n* (%) | | | 11 (31.4) | |
| UN, *n* (%) | | | | |
| ILD disease duration, years median (IQR) | | | 4 (1–6) | |
| Age at ILD diagnosis, years median (IQR) | | | 65.7 ± 10 | |

Data presented as number of patients (n) and percentage (%) in bracket or median and interquartile range in bracket. RA, Rheumatoid Arthritis; RF, Rheumatoid Factor; ACPA, anticitrullinated peptide antibodies; DAS28, Disease Activity Score on 28 joints; CRP, C-reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; bDMARDs, Biological disease modifying anti-rheumatic drugs; ILD, Interstitial Lung Disease; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; UN, unclassifiable.
Significant *p*-values are reported in bold. ns, not significant.

### 3.2. Telomere length

We evaluated the TL in 85 RA patients, 50 RA-NILD and 35 RA-ILD, and 21 CTRLs. As shown in Figure 1, TL was significantly shorter in all RA patients compared to CTRLs ($p = 0.003$). In particular, RA-ILD patients showed significantly shorter TL compared to both CTRLs ($p = 0.0001$) and RA-NILD ($p = 0.004$) (Figure 1). The most significant shortening of TL in RA-ILD was confirmed after multiple corrections for sex, age, disease duration and CRP (Padj= 0.0001).

Moreover, in RA-ILD patients TL negatively correlated with RA disease duration ($p = 0.004$, $R = -0.485$), but not with ILD duration (Figure 2). In addition, patients with UIP pattern, the most common and severe manifestation of ILD, exhibit the most evident shortening of TL compared to other RA patients ($p < 0.0001$), followed by those patients affected by UN-ILD ($p = 0.009$) (Figure 3).

After patients' stratification in "short" and "normal" TL (T/S ratio within or above the first quartile), we observed that the prevalence of ILD was significantly higher in patients with short- vs normal-TL (73.4 vs 31.8%, $p = 0.001$, and OR= 6.00).

### 3.3. Mitochondrial DNA copy number

We then evaluated the mtDNA copy number in the same cohorts of patients and controls. RA patients exhibited a

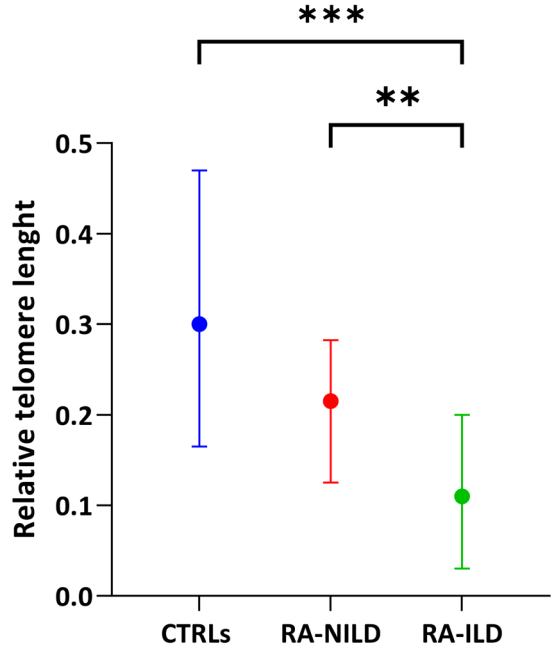


**Figure 1.** Comparison of relative TL among CTRLs, RA-NILD, and RA-ILD subjects.
***$p = 0.0001$; **$p = 0.004$.

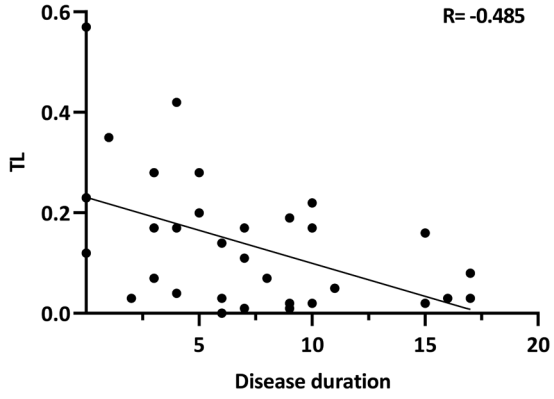


**Figure 2.** Correlation analyses between telomere length and disease duration in RA-ILD patients.
$p = 0.004$.

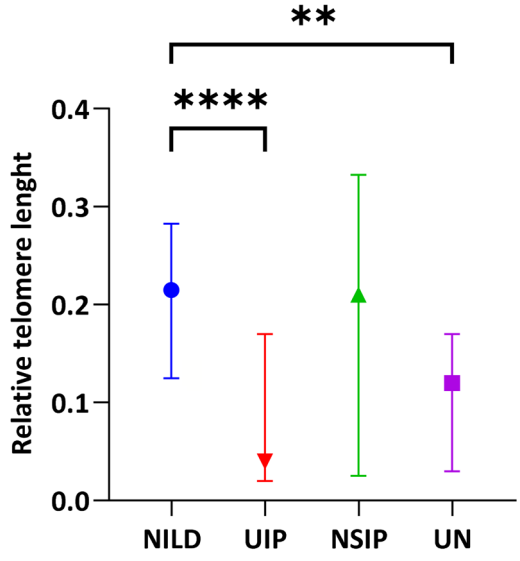


**Figure 3.** Comparison of relative TL between RA-NILD patients and RA-ILD patients with different patterns of lung involvement.
****$p < 0.0001$; **$p = 0.009$.

significantly lower amount of mtDNA copy number compared to CTRLs ($p < 0.0001$), also considering the two subgroups of RA-ILD and RA-NILD patients ($p = 0.04$ and $p = 0.0005$, respectively) (Figure 4), but no differences were observed between the two RA patients' subgroups. After patients' stratification according to the manifested pattern, no differences were observed among the subgroups.

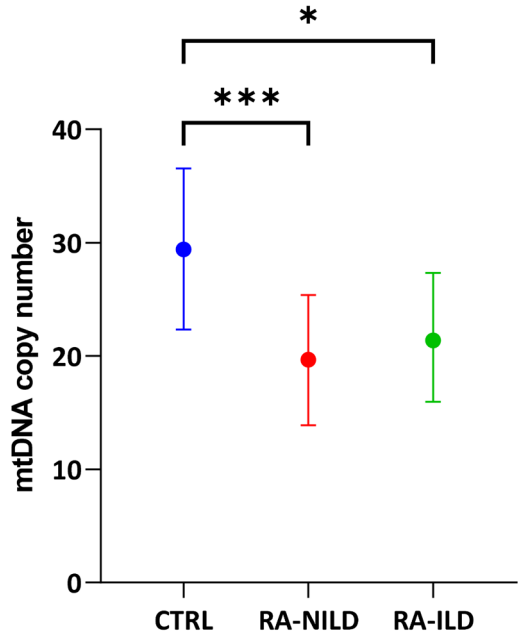


**Figure 4.** Comparison of mtDNA copy number among CTRLs, RA-NILD, and RA-ILD subjects.
***$p = 0.0005$; *$p = 0.04$.

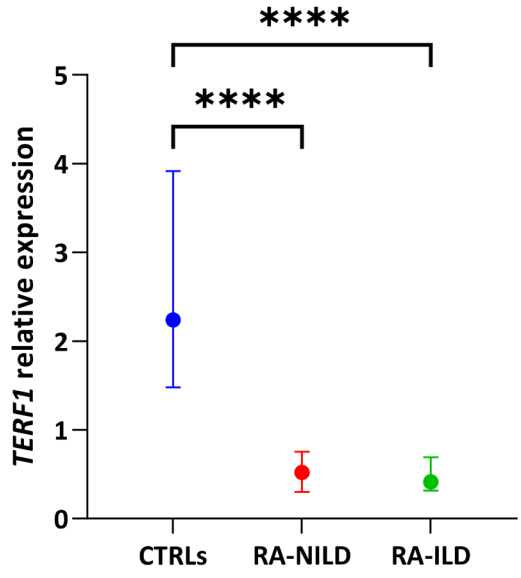


**Figure 5.** Comparison of *TERF1* expression levels among CTRLs, RA-NILD, and RA-ILD subjects.
****$p < 0.0001$; ****$p < 0.0001$.

### 3.4. Expression levels of regulator gene factors

We subsequently investigated the expression levels of genes involved in maintaining TL (*TERF1*) and mtDNA copy number (*TFAM*) in the previously analyzed subjects. *TERF1* expression levels were reduced in RA patients compared to CTRLs ($p < 0.0001$), both considering RA-ILD and RA-NILD patients ($p < 0.0001$ and $p < 0.0001$, respectively), but no differences were observed between the two patients' subgroups (Figure 5).

*TFAM* expression levels were reduced in RA patients compared with CTRLs ($p = 0.04$). Considering the two patients' subgroups separately, this decrease remains statistically significant only in the RA-ILD patients' subgroup ($p = 0.03$) (Figure 6). Moreover, we observed a difference in *TFAM* expression levels between RA-NILD and RA-ILD after multiple corrections for sex, age, disease duration, and CRP (Padj= 0.019).

## 4. Discussion

In this study, we have evaluated two of the main hallmarks of cellular senescence, telomere length and mitochondria

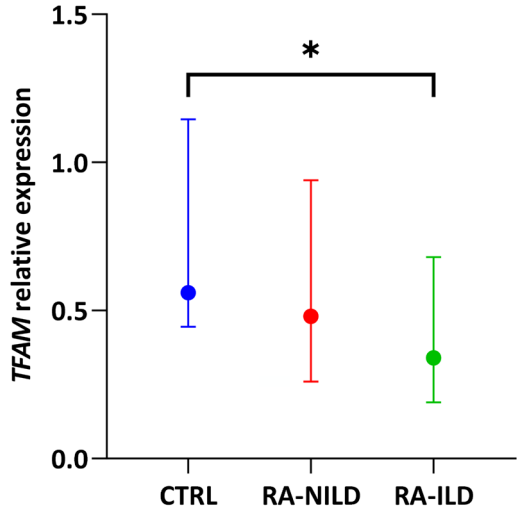


**Figure 6.** Comparison of *TFAM* expression levels among CTRLs, RA-NILD, and RA-ILD subjects.
*$p = 0.03$.

dysregulation, in a cohort of RA patients with and without lung involvement.

Cellular senescence is defined as the final and irreversible stage of cell cycle arrest [26]. It is strictly linked to OS and both are known to contribute together to several pathologies, including autoimmune and fibrotic pulmonary diseases [27,28].

Several studies have shown that leukocytes with shortened telomeres release inflammatory cytokines, which cause increased inflammation, leading to joint destruction and systemic symptoms [29,30]. It is known that telomeres erosion characterizes both lymphoid and myeloid cells in RA patients [31] and Zhen et al. have highlighted how cellular senescence actively participates in the pathogenesis and progression of RA [32]. However, a recent study described that shortened telomeres are also associated with ILD status among patients with RA [15]. Our data are consistent with this observation; in fact, we have shown an alteration of TL in RA patients, that is more serious in the presence of ILD complication. In addition, we have highlighted that the most evident shortening of TL is visible in those patients who present the most common and severe pattern UIP. This result agrees with the study by Joyce JS et al. which showed that telomeres were shorter in alveolar type II cells of UIP lungs compared to healthy controls [33].

The negative correlation between TL and RA disease duration underscores the importance of cumulative cellular damage in these patients. We can hypothesize that TL might be a biomarker of lung involvement and future studies on large cohort could help in the identification of a possible threshold of TL which is associated to the higher risk of ILD.

To evaluate if the telomere shortening in RA-ILD patients is associated with a dysregulation of the factors involved in the preservation of their integrity, we have evaluated the expression of *TERF1* gene. We found that both RA-ILD and RA-NILD patients have statistically lower mRNA levels for *TERF1* gene compared with the controls, but we did not observe differences between the two patients' subgroups. *TERF1* gene codes for a protein component of the shelterin complex that binds the telomere region and acts as a telomerase inhibitor [34]. Studies in literature are contrasting, but several authors have highlighted that a reduction of *TERF1* levels results in telomeric DNA fragility and damage [35,36]; in addition, *TERF1* deletion results in rapid induction of senescence, mediated by several pathways, suggesting a role for *TERF1* also in telomere protection [35].

Since the mtDNA copy number could change in response to the increased OS, we have decided to evaluate also the mtDNA copies as markers of cellular senescence in the same cohort of patients and controls. In addition, it is known that mitochondrial dysfunctions, in turn, promote the overproduction of OS and inflammatory mediators, typical of autoimmune disorders such as RA [37]. Our results show that RA patients exhibited a significantly lower amount of mtDNA copy number compared to controls. This data is in accordance with other studies that demonstrated a decrease in mtDNA copy number in this disorder compared to healthy controls [21,38]. In addition, for the first time, we have demonstrated that this dysregulation is present also in RA patients with ILD.

The mtDNA copy number is regulated by the activity of a mitochondrial transcription factor encoded by the *TFAM* gene. We have shown that *TFAM* expression levels were reduced in RA patients compared with CTRLs; in particular, we observed this decrease in the RA-ILD subgroup. Since it is known that this factor also protects and prevents damage of mtDNA from ROS [39], we could suppose that the decreased *TFAM* levels in RA patients participate in increased OS, leading to cellular senescence, in particular in those patients with ILD manifestation.

The presence of ILD in RA patients is associated with a more severe disease phenotype, with a worse prognosis in terms of morbidity and mortality. Therefore, the identification of those patients at risk to develop ILD is crucial for an early diagnosis and treatment. The combination of clinical and laboratory biomarkers together with screening tests (CT scan) in patients at high risk may result in early identification.

Our study has some limitations. Firstly, the cross-sectional design limits the ability to establish causal relationships. Moreover, the relatively small sample size might limit the generalizability of our results. Longitudinal studies could help verify if these biomarkers can predict the progression of ILD in RA patients. In addition, it will also be interesting to replicate the study on sub-populations of PBMCs.

In conclusion, our results highlight significant differences in cell senescence indicators between RA patients and CTRLs. Among these, the shortening of telomere is strongly evident in RA patients with lung involvement. Further studies are necessary to confirm these findings and explore potential role of these biomarkers in identifying patients at risk of developing ILD and in monitoring disease progression.

## Authors' contributions

Conceptualization: P.B., P.C.; Data curation: G.D.B., A.Lat.; Formal analysis: G.D.B., A.Lat., C.M.; Funding acquisition: M.S.C., G.N.; Investigation: G.D.B., A.Lat., C.M., C.B., E.C.; Resources: C.B., E.C.,

B.K., A.Luc., F.C., J.O., P.C.; Supervision: M.S.C., M.C., P.R., G.N.; Visualization: C.C., P.C.; Writing - original draft: G.D.B.; Writing - review & editing: A.Lat., C.C., P.C., P.B.

## Diclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Funding

This study was partially supported by MUR-PNRR M4-C2-I1.3 PE6 project PE00000019 Heal Italia [CUP: E83C22004670001] to G.N. and by European Union - NextGenerationEU: National Center for Gene Therapy and Drugs based on RNA Technology, CN3 - Spoke 7 (code: CN00000041) to M.S.C.

## References

[1] Kim EJ, Collard HR, King TE.Jr. Rheumatoid arthritis-associated interstitial lung disease: the relevance of histopathologic and radiographic pattern. Chest. 2009;136(5):1397–1405. doi: 10.1378/chest.09-0444

[2] Maher TM. Interstitial lung disease: a review. JAMA. 2024;331(19):1655–1665. doi: 10.1001/jama.2024.3669

[3] Flashner BM, VanderLaan PA, Nurhussien L, et al. Pulmonary histopathology of interstitial lung disease associated with antisynthetase antibodies. Respir Med. 2022;191:106697. doi: 10.1016/j.rmed.2021.106697

[4] Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(5):e44–e68. doi: 10.1164/rccm.201807-1255ST

[5] O'Callaghan M, Bonella F, McCarthy C. Unclassifiable, or simply unclassified interstitial lung disease? Curr Opin Pulm Med. 2021;27(5):405–413. doi: 10.1097/MCP.0000000000000801

[6] Kadura S, Raghu G. Rheumatoid arthritis-interstitial lung disease: manifestations and current concepts in pathogenesis and management. Eur Respir Rev. 2021;30(160):210011. doi: 10.1183/16000617.0011-2021

[7] Bilgici A, Ulusoy H, Kuru O, et al. Pulmonary involvement in rheumatoid arthritis. Rheumatol Int. 2005;25(6):429–435. doi: 10.1007/s00296-004-0472-y

[8] Gochuico BR, Avila NA, Chow CK, et al. Progressive preclinical interstitial lung disease in rheumatoid arthritis. Arch Intern Med. 2008;168(2):159–166. doi: 10.1001/archinternmed.2007.59

[9] Park HS, Kim SR, Lee YC. Impact of oxidative stress on lung diseases. Respirology. 2009;14(1):27–38. doi: 10.1111/j.1440-1843.2008.01447.x

[10] Liu X, Chen Z. The pathophysiological role of mitochondrial oxidative stress in lung diseases. J Transl Med. 2017;15(1):207. doi: 10.1186/s12967-017-1306-5

[11] Yadav S, Maurya PK. Correlation between telomere length and biomarkers of oxidative stress in human aging. Rejuvenation Res. 2022;25(1):25–29. doi: 10.1089/rej.2021.0045

[12] Alder JK, Chen JJ-L, Lancaster L, et al. Short telomeres are a risk factor for idiopathic pulmonary fibrosis. Proc Natl Acad Sci U S A. 2008;105(35):13051–13056. doi: 10.1073/pnas.0804280105

[13] Svyryd Y, Pascual-Ramos V, Contreras-Yañez I, et al. Telomeres length variations in a rheumatoid arthritis patients cohort at early disease onset and after follow-up. Rev Invest Clin. 2022;74(4):202–211.

[14] Natalini JG, England BR, Baker JF, et al. Associations between shortened telomeres and rheumatoid arthritis-associated interstitial lung disease among U.S. Veterans. Respir Med. 2022;201:106943. doi: 10.1016/j.rmed.2022.106943

[15] Doyle TJ, Juge P-A, Peljto AL, et al. Short peripheral blood leukocyte telomere length in rheumatoid arthritis-interstitial lung disease. Thorax. 2024;79(2):182–185. doi: 10.1136/thorax-2023-220022

[16] Correia-Melo C, Marques FDM, Anderson R, et al. Mitochondria are required for pro-ageing features of the senescent phenotype. EMBO J. 2016;35(7):724–742. doi: 10.15252/embj.201592862

[17] Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci U S A. 1997;94(2):514–519. doi: 10.1073/pnas.94.2.514

[18] Kowalski S, Wyrzykowski D, Inkielewicz-Stępniak I. Molecular and cellular mechanisms of cytotoxic activity of vanadium compounds against cancer cells. Molecules. 2020;25(7):1757. doi: 10.3390/molecules25071757

[19] Kang I, Chu CT, Kaufman BA. The mitochondrial transcription factor TFAM in neurodegeneration: emerging evidence and mechanisms. FEBS Lett. 2018;592(5):793–811. doi: 10.1002/1873-3468.12989

[20] Noack H, Bednarek T, Heidler J, et al. TFAM-dependent and independent dynamics of mtDNA levels in C2C12 myoblasts caused by redox stress. Biochim Biophys Acta. 2006;1760(2):141–150. doi: 10.1016/j.bbagen.2005.12.007

[21] Svendsen AJ, Tan Q, Jakobsen MA, et al. White blood cell mitochondrial DNA copy number is decreased in rheumatoid arthritis and linked with risk factors. A twin study. J Autoimmun. 2019;96:142–146. doi: 10.1016/j.jaut.2018.09.008

[22] Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative [published correction appears in Ann Rheum Dis. 2010 Oct;69(10):1892]. Ann Rheum Dis. 2010;69(9):1580–1588. doi: 10.1136/ard.2010.138461

[23] Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res. 2002;30(10):e47–47. doi: 10.1093/nar/30.10.e47

[24] Rooney JP, Ryde IT, Sanders LH, et al. PCR based determination of mitochondrial DNA copy number in multiple species. Methods Mol Biol. 2015;1241:23–38. doi: 10.1007/978-1-4939-1875-1_3

[25] Xing J, Chen M, Wood CG, et al. Mitochondrial DNA content: its genetic heritability and association with renal cell carcinoma. J Natl Cancer Inst. 2008;100(15):1104–1112. doi: 10.1093/jnci/djn213

[26] Huang W, Hickson LJ, Eirin A, et al. Cellular senescence: the good, the bad and the unknown. Nat Rev Nephrol. 2022;18(10):611–627. doi: 10.1038/s41581-022-00601-z

[27] Lu Y, Ruan Y, Hong P, et al. T-cell senescence: a crucial player in autoimmune diseases. Clin Immunol. 2023;248:109202. doi: 10.1016/j.clim.2022.109202

[28] Hernandez-Gonzalez F, Pietrocola F, Cameli P, et al. Exploring the interplay between cellular senescence, immunity, and fibrosing interstitial lung diseases: challenges and opportunities. Int J Mol Sci. 2024;25(14):7554. doi: 10.3390/ijms25147554

[29] O'Donovan A, Pantell MS, Puterman E, et al. Cumulative inflammatory load is associated with short leukocyte telomere length in the health, aging and body composition study. PLoS One. 2011;6(5):e19687. doi: 10.1371/journal.pone.0019687

[30] Lustig A, Liu HB, Metter EJ, et al. Telomere shortening, inflammatory cytokines, and anti-cytomegalovirus antibody follow distinct age-associated trajectories in humans. Front Immunol. 2017;8:1027. doi: 10.3389/fimmu.2017.01027

[31] Colmegna I, Weyand CM. Haematopoietic stem and progenitor cells in rheumatoid arthritis. Rheumatology (Oxford). 2011;50(2):252–260. doi: 10.1093/rheumatology/keq298

[32] Zeng Z, Zhang W, Qian Y, et al. Association of telomere length with risk of rheumatoid arthritis: a meta-analysis and Mendelian randomization. Rheumatology (Oxford). 2020;59(5):940–947. doi: 10.1093/rheumatology/kez524

[33] Lee JS, La J, Aziz S, et al. Molecular markers of telomere dysfunction and senescence are common findings in the usual interstitial pneumonia pattern of lung fibrosis. Histopathology. 2021;79(1):67–76. doi: 10.1111/his.14334

[34] Chan SR, Blackburn EH. Telomeres and telomerase. Philos Trans R Soc Lond B Biol Sci. 2004;359(1441):109–121. doi: 10.1098/rstb.2003.1370

[35] Martínez P, Thanasoula M, Muñoz P, et al. Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. Genes Dev. 2009;23(17):2060–2075. doi: 10.1101/gad.543509

[36] Hohensinner PJ, Kaun C, Buchberger E, et al. Age intrinsic loss of telomere protection via TRF1 reduction in endothelial cells. Biochim Biophys Acta. 2016;1863(2):360–367. doi: 10.1016/j.bbamcr.2015.11.034

[37] Ma C, Wang J, Hong F, et al. Mitochondrial dysfunction in rheumatoid arthritis. Biomolecules. 2022;12(9):1216. doi: 10.3390/biom12091216

[38] Zhan D, Tanavalee A, Tantavisut S, et al. Relationships between blood leukocyte mitochondrial DNA copy number and inflammatory cytokines in knee osteoarthritis. J Zhejiang Univ Sci B. 2020;21(1):42–52. doi: 10.1631/jzus.B1900352

[39] Kang D, Kim SH, Hamasaki N. Mitochondrial transcription factor A (TFAM): roles in maintenance of mtDNA and cellular functions. Mitochondrion. 2007;7(1-2):39–44. doi: 10.1016/j.mito.2006.11.017