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Early View

Research letter

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Leukocyte Telomere Length and Mycophenolate Therapy in Chronic Hypersensitivity Pneumonitis

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Abbreviation List

CI = Confidence Interval; CHP= chronic hypersensitivity pneumonitis; DLCO = diffusion capacity of the lung for carbon monoxide; FVC = forced vital capacity; HR = hazard ratio; HRCT = high-resolution computed tomography; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; IRB = institutional review board; MMF=mycophenolate mofetil; PFT = pulmonary function testing; SD = standard deviation; UIP = usual interstitial pneumonia

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Conception and design: AA, JM, JMO, and BL

Acquisition of data for the work: AA, JM, CN, EV, ALL, PJW, MES, IN, CKG, JMO, and BL

Analysis and interpretation: AA, JM, CN, EV, JMO, and BL

Drafting the manuscript for important intellectual content: AA, JM, CN, EV, AL, PJW, MES, IN, CKG, JMO, and BL

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To the Editor:

Recent prospective clinical trials have shown antifibrotic therapies slow lung function decline in patients with idiopathic pulmonary fibrosis (IPF)[1,2] and progressive fibrosing interstitial lung disease (ILD). Similar findings were demonstrated in scleroderma-associated ILD[3] despite use of the immunosuppressive therapy mycophenolate mofetil (MMF). Prospective data for the treatment of other forms of ILD, such as chronic hypersensitivity pneumonitis (CHP) are lacking. Our groups previously reported that the treatment of CHP with MMF was associated with a decreased incidence of adverse events, a reduction in prednisone dose, and improved lung function when compared to prednisone alone[4,5], but prospective studies are needed to confirm these findings. Short leukocyte telomere length (TL) is associated with increased mortality in patients with ILD, including CHP and IPF[6-8]. A recent investigation also showed TL may influence the response to immunosuppressive therapy. In that study, patients with IPF and short TL had a higher risk of death, lung transplantation, and forced vital capacity (FVC) decline, when exposed to immunosuppressive therapy, including MMF[9]. In this investigation we sought to determine whether similar findings occurred in patients with CHP. We hypothesized that patients with CHP and short TL would experience a higher prevalence of death and disease progression when compared to those with longer TL.

The study population consisted of a multicenter cohort of prospectively enrolled consenting patients with a confident multidisciplinary diagnosis of CHP at the University of Chicago (UChicago), University of California San Francisco (UCSF), University of California Davis (UCDavis), and University of Texas Southwestern, Dallas (UTSW) between September 2003 and December 2019 (IRB: UC#14163A, UCSF#10-01592 & #10-00198; UCD#585448-7 & #875917-2, UTSW#082010-127; #AAAS0753). Genomic DNA was isolated from peripheral blood leukocytes, TL measurement performed using quantitative PCR in triplicate[10], and age-adjusted TL calculated using normal controls. Standardized TLs were obtained by normalization across study sites and categorized into quartiles. The electronic medical record was used to extract pertinent clinical information and determine vital status, which was confirmed using the US Social Security death index. Patients who received azathioprine prior to or during the study period were excluded (n=19). A binary categorization was applied to the study population based on MMF therapy ≥ 500mg/day for at least a month during the study period.

A propensity score approach was utilized to predict the conditional probability for an individual to receive MMF treatment, and model covariates included: age, sex, smoking status, prednisone therapy, physiologic indices of disease severity such as FVC and diffusing capacity of the lung for carbon monoxide (DLCO), severity of fibrosis, distribution of fibrosis, traction bronchiectasis, UIP pattern, ground-glass opacities, and mosaic attenuation. Inverse probability of treatment weighting (IPTW) was used to estimate the average treatment effect on time-to-event outcomes[11]. Survival functions were plotted using the Kaplan-Meier estimator. Cox models were used for hazard ratio estimation calculating transplant-free survival time as time from commencing immunosuppressive therapy to death, lung transplantation, loss to follow-up, or end of study period, while adjusting for imbalanced variables and controlling for center in all multivariable outcome models. We applied multiple imputation using chained equations to account for missing covariates (<20%). IPTW-weighted longitudinal trajectories of FVC%

predicted, and DLCO% predicted were analyzed using linear mixed-effects models with restricted maximum likelihood modeling and an autoregressive structure[4], and grouped PFTs into 90-day epochs allowing for time-course alignment all patients. The change in FVC% predicted, and DLCO% predicted was evaluated, and characteristics compared using two-sided t-tests, or chi-square tests, as appropriate. Statistical analyses were conducted using Stata (2019.R.16; StataCorp).

In this investigation, 208 patients with CHP were enrolled, of which 19 were excluded because they had received azathioprine before or during the study period. The remaining 189 patients with 1,420 unique PFTs were included. Median age was 65 years (interquartile range, IQR 58–71 years), 97 (51%) were female, 160 (85%) were white, 89 (47%) had a history of tobacco use and a mean of 15±22 pack years, and 129 (68%) had a history of environmental antigens. Baseline FVC% predicted and DLCO% predicted were 65%±19% and 53%±23%, respectively, 99 (52%) patients had undergone surgical lung biopsy, and 142 (75%) received corticosteroids. Clinical characteristics across study centers are summarized in Fig. 1A. Median MMF exposure time was similar between patients with TL in the first quartile (Q1) and those in the second to fourth quartiles (Q2-Q4) (10months (IQR=5-16months) vs. 10months (IQR=4-23months); *P*=0.86). Use of corticosteroid therapy was similar between both groups (Q1=77.1% vs. Q2-Q4=74.5%; *P*=0.72). Baseline FVC% predicted was lower in Q1 patients that received MMF when compared to those that did not receive MMF (63.2%±18.2% vs. 74.1%±14.7%; *P*=0.029). Similarly, baseline DLCO% predicted was lower in Q2-Q4 patients who received MMF than those that did not receive MMF (48.2%±20.3% vs. 58.2%±24.4%; *P*=0.011).

Each quartile decrease in TL was associated with a step-wise decrease in transplant-free survival (Fig.1B). Crude mortality rates were higher for Q1 patients when compared to Q2-Q4 (27.3 deaths per 100 person-yrs vs. 8.4 deaths per 100 person-yrs; *P*=0.0002). In propensity score adjusted analyses Q1 patients had increased mortality overall when compared to Q2–Q4 (HR, 3.29; 95%CI, 1.56–6.95; *P*=0.002). When compared to Q1 patients who did not receive MMF, survival was improved in Q2–Q4 patients who received MMF (HR for interaction term, 0.17; 95%CI; 0.05-0.61; *P*=0.007), but not in Q2–Q4 patients who did not receive MMF (*P*=0.13), or in Q1 patients who received MMF (*P*=0.87) (Fig.1C-D). Significant interaction existed between MMF and TL for Q3 (HR, 0.19; 95%CI, 0.05–0.70; *P*=0.013) and Q4 (HR, 0.18; 95%CI, 0.06–0.57; *P*=0.003), but not for Q1 (*P*=0.72) or Q2 (*P*=0.37). Seven patients were censored due to lung transplantation, all with TL above the first quartile. Those who received MMF appeared more likely to undergo lung transplantation (n=6; 10%) compared to those who did not receive MMF (n=1, 2%; *P*=0.066). Importantly, irrespective of TL, annualized change in FVC and DLCO measurements did not differ with MMF use (Fig.1E-F).

Our observation that MMF therapy is not associated with improved survival or lung function in patients with CHP and short telomeres is similar to the association in IPF, and likely reflects a final common pathway in the pathophysiologic processes of advanced fibrosis that underlie these two diseases. This is further evidenced by the demonstrated benefit of antifibrotic therapy in reducing lung function decline for patients with IPF or CHP, with increasing evidence of the benefit of nintedanib and pirfenidone in progressive fibrosis irrespective of TL[12,13]. As patients with CHP are often prescribed immunosuppressive medications, increased recognition of the

potentially harmful effects of these therapies in IPF subjects with short telomeres has engendered increased scrutiny around their use in other types of pulmonary fibrosis.

In the absence of short TL, the improved survival associated with MMF in CHP may suggest fundamental differences between IPF and CHP. While IPF is not characterized by inflammation, CHP has an inflammatory component, and immunosuppressive therapy may ameliorate the exposure-related alveolar inflammation earlier in the disease course. Additionally, while specific genetic variants are common to both diseases, the repertoire of telomere-related mutations and susceptibility associated gene polymorphisms such as MUC5B appear to differ in their associations with lung function decline and survival across different types of ILD[8,14]. Of note, in this observational study, we restricted our analysis to assessing the influence of MMF on CHP outcomes, and did not evaluate potential adverse effects such as hematological and hepatic effects. Thus, the retrospective nature of this study limits our findings to the identification of association, not causality; however, the consistency of our findings with previous investigations supports these results.

Ultimately, as the management of CHP continues to evolve, larger carefully conducted studies examining the value of immunosuppressive therapy in patients with telomere mutation–related ILD remain much needed given the widespread use of these medications and their presumed value in diverse forms of ILD.

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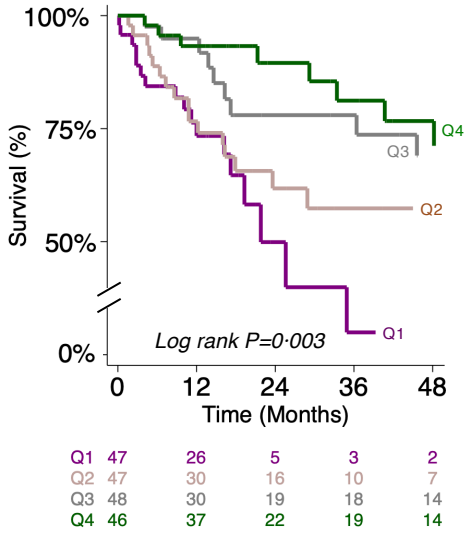
A.

Parameter	UCHICAGO (n=78)	UCDAVIS (n=49)	UCSF (n=35)	UTSW (n=27)
Age, yr	65 (9)	71 (10)	60 (11)	62 (9)
Male	39 (50)	23 (47)	14 (40)	16 (59)
White	66 (85)	40 (82)	30 (86)	24 (89)
Tobacco pk-yrs	16 (24)	11 (18)	17 (13)	16 (27)
Env. Antigen	24 (31)	49 (100)	35 (100)	21 (78)
FVC %	65 (20)	66 (20)	71 (15)	59 (19)
DLCO %	55 (25)	52 (24)	58 (13)	45 (19)
Surgical biopsy	43 (55)	15 (31)	25 (71)	16 (59)
Corticosteroid	54 (69)	38 (78)	26 (74)	24 (89)

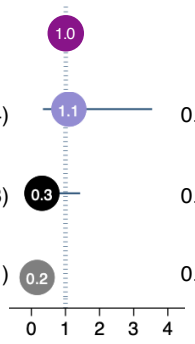
B.



C.

Propensity-score adjusted Cox regression model

Telomere Length	Hazard ratio (95% CI)	*P*-value
Q1, - MMF	*reference*	--
Q1, + MMF	1.10 (0.34 – 3.54)	0.869
Q2-Q4, - MMF	0.31 (0.07 – 1.43)	0.133
Q2-Q4, + MMF	0.17 (0.05 – 0.61)	0.007



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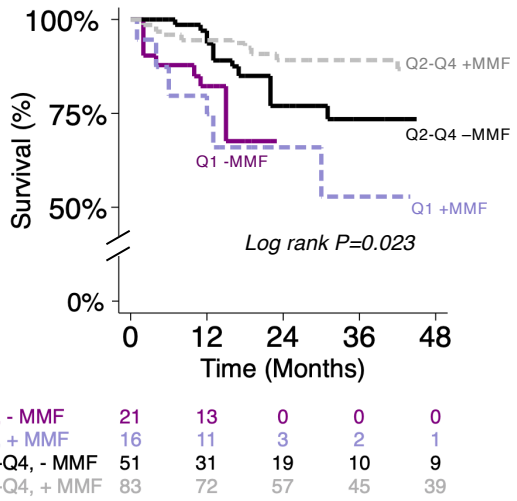

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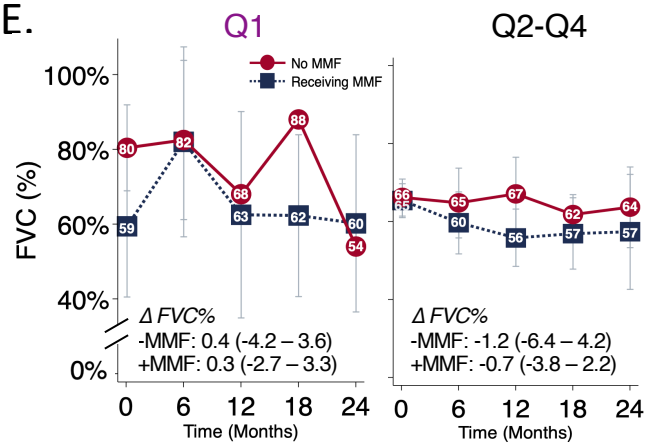

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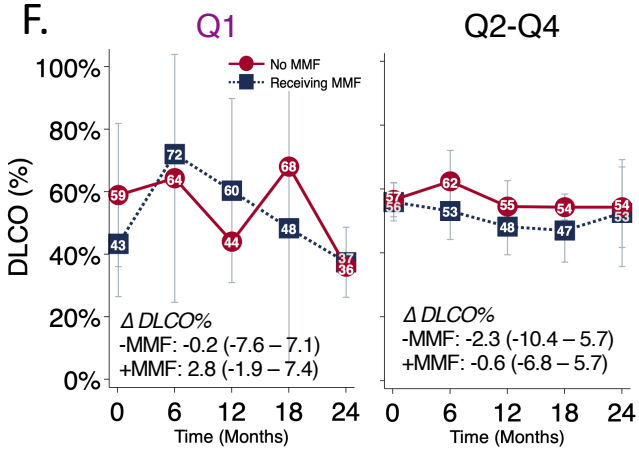

Figure 1. (A) Baseline characteristics of hypersensitivity pneumonitis (HP) study population. **(B)** Transplant-free survival in patients with HP according to quartiles of telomere length (TL)*. **(C)** Propensity score adjusted Cox regression model (reference= Q1 patients not receiving mycophenolate therapy [MMF]). **(D)** Propensity score adjusted transplant-free survival. **(E)** Percentage predicted forced vital capacity (FVC) trajectory from baseline for the first 24 months grouped by MMF therapy *(blue squares and dotted lines)* vs. no MMF therapy *(red circles and solid lines)* for first quartile (Q1), and second to fourth quartiles (Q2-Q4) of TL. **(F)** Percent predicted diffusing capacity (DLCO) trajectory from baseline for the first 24 months grouped by MMF therapy vs. no MMF therapy for patients with TL in Q1, and Q2-Q4. *Δ = annualized change in pulmonary function test (PFT). Categorical variables presented as n (%); continuous variables presented as mean (SD). UCHICAGO=University of Chicago, UCDAVIS=University of California, Davis, CA; UCSF=University of California, San Francisco, CA; UTSW=University of Texas Southwestern Medical Center, Dallas, TX. Env.Antigen=environmental antigen; Tobacco pk-yrs=tobacco pack years; surgical biopsy=surgical lung biopsy; corticosteroid=prednisone use. *One subject was censored after baseline evaluation as survival status could not be ascertained during the follow-up evaluation period. Whiskers correspond to 95% confidence intervals. Median time of 1st MMF dose =1.4 months from 1st PFT. Median time of last MMF dose =11.5 months from 1st PFT. Longitudinal analysis restricted to subjects with ≥2 PFTs over the first 24 months of study period.*