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Deposited on: 27 October 2017
Patients with gout have short telomeres compared with healthy participants; association of telomere length with flare frequency and cardiovascular disease in gout

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Keywords: Gout, telomere, senescence, inflammation, cardiovascular disease
Abstract:

Aim and background: Chronic inflammation associates with increased senescence, which is a strong predictor for cardiovascular disease. We hypothesised that inflammation accelerates senescence and thereby enhances the risk of cardiovascular disease in gout.

Methods: We assessed replicative senescence by quantifying telomere length (TL) in a discovery cohort of 145 Dutch patients with gout and 273 healthy individuals and validated our results in 474 patients with gout and 293 healthy participants from New-Zealand. Subsequently, we investigated the effect of cardiovascular disease on TL of all participants. Also, we measured TL of CD4\(^+\) and CD8\(^+\) T lymphocytes, B lymphocytes, monocytes, natural killer (NK) cells and plasmacytoid dendritic cells (pDCs). Additionally, we assessed the potential temporal difference in TL and telomerase activity.

Results: TL in PBMCs of healthy donors decreased over time reflecting normal ageing. Patients with gout demonstrated shorter telomeres \((P=0.001, R^2=0.01873)\). In fact, the extent of telomere erosion in patients with gout was higher at any age as compared to healthy counterparts at any age \((P<0.0001, R^2=0.02847)\). Patients with gout with cardiovascular disease had the shortest telomeres and TL was an independent risk factor for cardiovascular disease in patients with gout \((P=0.001)\). TL was inversely associated with the number of gouty flares \((P=0.005)\).

Conclusions: Patients with gout have shorter telomeres than healthy participants, reflecting increased cellular senescence. Telomere shortening was associated with the number of flares, and with cardiovascular disease in people with gout.
Introduction

Gout is a rheumatic disease characterized by deposition of monosodium urate (MSU) crystals in and around the joints, associated with elevated serum urate levels. Gout is the most common inflammatory arthritis in men older than 40 years and it affects 1-5% of adults in the Western populations [1–5]. Patients experience recurrent episodes of acute joint inflammation, and, in the presence of persistent hyperuricemia, may also develop chronic joint inflammation. We recently demonstrated that patients with gout display high levels of inflammatory mediators such as IL-8 even though these patients did not have clinically apparent inflammatory disease at the time of sample collection [6].

Chronic and recurrent inflammation activates the immune system, elevating the demand for immune cells, damaging tissues and forcing cell replication. This can ultimately eventuate a state of replicative senescence in most cells. Replicative senescence is when cells can no longer divide to form new cells. These senescent cells secrete pro-inflammatory markers such as IL-6 and IL-1α[7]. The most commonly used marker for replicative senescence is telomere length (TL). Telomeres function as a non-coding protective end-region of chromosomes. Due to the DNA end-replication inefficiency of polymerases, chromosomes shorten every cell division. After a certain number of divisions, the threshold of attrition is reached, cell-cycle arrest occurs and eventuates the senescent state. Telomeres therefore are indicative of the number of divisions the cell lineage has undergone and are a measure of replicative senescence.

Since recurrent and chronic inflammation leads to replicative senescence, which in turn contributes to a pro-inflammatory environment, measuring replicative senescence by TL has been a subject of scrutiny in inflammatory diseases[8]. Telomere shortening has been repeatedly associated with multiple inflammatory diseases. Previously, TL has been associated with lifespan, although this remains controversial, and with cardiovascular disease[9]. The latter association has been recently substantiated in a large meta-analysis including over 50,000 individuals[10]. This study showed a significantly increased relative risk for developing cardio- and
cerebrovascular disease in the tertile of the population with the shortest telomeres. Causality between senescence and cardiovascular diseases is supported by the presence of a high number of senescent cells in atherosclerotic plaques, enhancing inflammation and decreasing recovery potential. Endothelial cells, vascular smooth muscle cells (VSMC) and immune cells are involved. The reduced proliferative capacity of endothelial cells and VSMCs, together with the pro-inflammatory environment lead to plaque instability, preluding vascular events[11–13].

Gout is often accompanied by cardiovascular comorbid conditions including myocardial infarction and cerebrovascular events, which lead to an increased morbidity and decreased life span[14,15]. Gout is also associated with metabolic syndrome, diabetes and renal failure, which in turn are predictors for cardiovascular disease. However, patients with gout remain at higher risk for developing cardiovascular disease after correction for these variables[16].

We hypothesize that chronic and recurrent acute inflammation in gout accelerates replicative senescence, increasing the risk of cardiovascular disease. That is, patients with gout with the highest burden of replicative senescence, will also have the highest burden of cardiovascular disease. To test our hypothesis, we investigated the most studied hallmark of replicative senescence, TL of PBMCs in two independent cohorts consisting of patients with gout and healthy individuals, both with and without cardiovascular disease. Also, we investigated telomere dynamics in various immune cell-subsets, scrutinized TL in a follow-up cohort and quantified hTERT (encoding human telomere reverse transcriptase) gene expression.
Methods

**Discovery cohort and validation cohort**

In our discovery cohort, we compared TL of PBMCs in 145 patients with gout with 273 Dutch participants (Supplementary files, table 1). In our validation cohort, we assessed TL in PBMCs collected from 474 Caucasian patients with gout and 293 healthy participants participating from New Zealand (Supplementary files, table 1).

This study was performed according to the guidelines of the Declaration of Helsinki and meets the ethical approval of the Ethical and Review committee of the University Medical Centre of Utrecht, Rijnstate hospital in Arnhem (both Netherlands) and the Multi-Region Ethics Committee in New Zealand. All the participants in this study gave their informed consent to participate.

**Isolation of mononuclear immune cell-subsets**

Mononuclear cell-subsets were sorted from total PBMCs isolated by Ficoll (Ficoll-Paque Plus, GE Healthcare) from peripheral blood of 10 Dutch patients with gout and 11 healthy participants (Supplementary files, table 3). Sorting the mononuclear cell-subsets was performed using fluorescence activated cell sorting (FACS) (FACSAria_III, BD Biosciences) to separate the cells according to the expression of cellular surface molecules; CD3+/CD56-/CD4+ for T helper lymphocytes, CD3+/CD56-/CD8+ for cytotoxic T lymphocytes, CD19+/CD20+ for B lymphocytes, CD14+ monocytes, CD3-/CD56+ NK cells and CD123 (IL3RA)+ / CD304 (BDCA4)+pDCs.

**Telomere Length Measurements**

DNA of PBMCs was extracted using a standard salting-out protocol. Absolute TL quantification[17] modified with synthetic standards as described by O’Connell et al., was performed using the Quantitative Polymerase Chain Reaction (q-PCR) method[18].

**Human telomerase reverse transcriptase (hTERT) gene expression**
Human telomerase reverse transcriptase (hTERT) gene expression level was quantified using synthesized cDNA (Biorad iScript kit) from RNA that was extracted from the PBMCs. Patients with gout (N=64) and healthy participants (N=89) from the Dutch cohort were randomly chosen. Quantstudio QPCR apparatus with Taqman assay (Applied Biosystems) were used to perform QPCR under conditions as specified by the manufacturer. To normalize the hTERT gene expression, the housekeeping GUSB and GAPDH genes were included. A similar method was used to assess hTERT gene expression in 10 patients with gout and 11 healthy participants immune cell subtypes separately.

**Statistical analyses**

For statistical analysis, IBM SPSS Statistics v23 (SPSS, Chicago, IL; 60606 U.S.A.) and Graphpad Prism v6 (GraphPad Software, San Diego, California) applications were applied. TL (in kilobase pairs) of patients with gout and healthy participants were log-transformed to normalize the distribution. A two-tailed T test was used, when appropriate with Welch' correction for comparing the unequal variances between the groups. Three independent linear regression models were applied to separately estimate: (A) the interpolation between TL and age, gender, smoking, cardiovascular disease, heart failure, stroke (non-fatal), MI (non-fatal), angina pectoris, systolic and diastolic blood pressure, BMI (kg/m²), serum UA level (mmol/L) and creatinine level (µmol/L) in patients and healthy individuals, (B) the association between TL and the presence of cardiovascular events (heart failure, stroke (non-fatal), MI (non-fatal), angina pectoris and age severally in individuals with gout and (C) the relation between TL and gout hallmarks including attack frequency, age at the first attack, disease duration, serum UA level (mmol/L), creatinine level (µmol/L) severally in patients group (Table 2).

In addition, the difference in TL between gout patients with or without cardiovascular events (A) and separately the association between flare frequency and cardiovascular events (angina pectoris, heart failure, myocardial infarction (non-fatal) and stroke (non-fatal)) (B) are represented in supplementary files, table 4. Univariate analysis was performed to examine the
effect of variables such as gender, age, smoking, BMI and creatinine levels on TL (Supplementary file, table 5). Where appropriate, two-tailed Pearson or Spearman bivariate correlations were applied to assess the correlation between the variables ($P<0.05$). The Bonferroni correction was applied to account for multiple testing.
Results

Demographics

Baseline characteristics of participants from the Netherlands and New Zealand are summarised in table 1.

Table 1. Baseline characteristics of Dutch and New Zealand patients and healthy participants.

<table>
<thead>
<tr>
<th></th>
<th>Gout (n=619)</th>
<th>Healthy (n=566)</th>
<th>participants</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male n (%)</td>
<td>489 (79.0)</td>
<td>398 (70.3)</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>62.8 ± 14.15</td>
<td>59.6 ± 13.67</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Age at the first flare (mean ± SD)</td>
<td>51.12 ± 16.75</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Disease duration (year) (mean ± SD)</td>
<td>12.9 ± 12.28</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Colchicine (yes) n (%)</td>
<td>254 (50.5)</td>
<td>1 (0.17)</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>NSAID (yes) n (%)</td>
<td>353 (75.5)</td>
<td>2 (0.35)</td>
<td>0.433</td>
<td></td>
</tr>
<tr>
<td>Allopurinol (yes) n (%) (mean 200mg/day)</td>
<td>337 (67.1)</td>
<td>1 (0.17)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids (yes) n (%)</td>
<td>197 (40.2)</td>
<td>1 (0.17)</td>
<td>0.643</td>
<td></td>
</tr>
<tr>
<td>CVD (yes/no) n (%)</td>
<td>270 (43.6)</td>
<td>43 (7.59)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Diabetes (type 2) (yes) n (%)</td>
<td>87 (14.0)</td>
<td>19 (3.36)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Stroke (yes/no) n (%)</td>
<td>43 (6.95)</td>
<td>67 (11.83)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction (non-fatal) (yes) n (%)</td>
<td>70 (8.6)</td>
<td>66 (11.6)</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>Heart failure (yes) n (%)</td>
<td>70 (11.3)</td>
<td>64 (11.31)</td>
<td>0.270</td>
<td></td>
</tr>
<tr>
<td>Angina pectoris (yes) n (%)</td>
<td>37 (5.98)</td>
<td>7 (1.24)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Creatinine level (µmol/L) (mean ± SD)</td>
<td>105.73 (± 39.38)</td>
<td>93.44 (± 30.29)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²) (mean ± SD)</td>
<td>29.73 (± 5.60)</td>
<td>28 (±6.37)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Smoking (yes) n (%)</td>
<td>51 (8.24)</td>
<td>5 (0.90)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Serum urate (mmol/L) (mean ± SD)</td>
<td>0.43 (± 0.13)</td>
<td>0.35 (± 0.10)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Total number of flares per year (mean ± SD)</td>
<td>8.75 (± 12.67)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Presence of tophi (yes/no) n (%)</td>
<td>116 (21.4)</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg) (mean ± SD)</td>
<td>139.90 (± 20.55)</td>
<td>136 (± 17.64)</td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg) (mean ± SD)</td>
<td>80.40 (± 11.83)</td>
<td>80.00 (± 9.62)</td>
<td>0.559</td>
<td></td>
</tr>
<tr>
<td>Telomere length (total leukocytes) (mean ± SD)</td>
<td>5924.90 (±8617.76)</td>
<td>8242.70 (± 8482.00)</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>
The data are presented as mean ± SD. The significance of the association between the 2 classified subgroups of gout and healthy participants was tested using Fisher’s exact test (categorical values) and Mann–Whitney U test (non-parametrical continues values) \( (P<0.05) \).

**PBMCs from patients with gout have shorter telomeres compared to their healthy counterparts in two unrelated cohorts**

Within our Dutch discovery cohort, we analysed the TL of PBMCs of 273 healthy participants and 145 patients with gout and correlated the results with their age in year. The TL in patients with gout was significantly shorter than healthy participants (supplementary files, table 1). In patients with gout, linear regression analyses demonstrated no association between age and TL \( (P>0.05, \ R^2=0.002853) \) (telomere shortening=0.002082*age+4.01). This was in contrast to the significant inverse/negative correlation between TL and age in healthy participants \( (P<0.0001, \ R^2=0.3721) \) (Telomere shortening=-0.02180*age+5.31) (Supplementary figure 1).

Next our results in the independent cohort of NZ Caucasian, showed that TL was significantly shorter in patients with gout \( (N=474) \) as compared to healthy participants \( (N=293) \) (supplementary table 2). Moreover, we found a negative association between TL and age in gout patients \( (P=0.0014, \ R^2=0.02276) \) (Telomere erosion= -0.004926*age+3.51). In healthy participants, we found a negative association with age \( (P<0.0001, \ R^2=0.111) \) (Telomere erosion=-0.01089*age+3.94) (Supplementary files, table 2) (Supplementary figure 2).

Given the similarities in TL of PBMCs in both the discovery and replication cohort, we pooled the data (Figure 1). When corrected for age, patients with gout have significantly shorter telomeres in PBMCs as compared to healthy participants \( (P<0.0001) \). TL correlates negatively with age in patients with gout \( (P=0.0010, \ R^2=0.01873) \) (TL=-0.006084*X+3.79). Similarly, PBMCs TL in healthy participants was inversely correlated with age \( (P<0.0001, \ R^2=0.02847) \) (TL=-0.006349*X+3.99).

Finally, we had access to follow-up data on TL from 9 patients with gout involved in the Dutch cohort of whom 88.90% were male of 46 to 87 years old with a median age of 63 years. The time period between the inclusion date and the follow-up at the second time-point was from 1 to 11
month with an average of 4.33 months. There was no (P=0.34) change in TL of the same patients with gout measured at two different time points (T1=4.20Kbp and T2=4.29Kbp).

**Gout and cardiovascular disease have an independent and cumulative effect on telomere attrition**

Since gout is strongly associated with cardiovascular disease, and cardiovascular disease has been associated with shorter telomeres, we investigated their separate effects. We included both patients with gout and participants with chronic heart disease (CHD), non-fatal myocardial infarction, non-fatal stroke and angina pectoris (Figure 2). Consistent with previous reports, healthy participants without cardiovascular disease had longer telomeres (P=0.04) as compared to healthy participants with cardiovascular disorders. The presence of cardiovascular disease in patients with gout resulted in significantly shorter telomeres (P=0.0001) compared to individuals with gout only. Moreover, TL in patients with gout without cardiovascular disease was shorter (P=0.0037) as compared to matched healthy individuals (Figure 2). Consequently, both gout and cardiovascular disease eventuated even shorter telomeres compared to healthy participants (P=0.005). Patients with cardiovascular disease concomitant with gout, demonstrated shorter telomeres (P<0.0001) as compared to healthy individuals without any comorbidities.

In the linear regression model involving both patients with gout and healthy individuals, gout (P=0.008), cardiovascular disease (P=0.023) and heart failure (P=0.01) were independently influencing TL (Table 2A). Of note, although all the continuous variables mentioned in table 2 were included in this model, gout, cardiovascular disease and heart failure remained in the model as significantly associated with TL. Moreover, in a linear regression model, cardiovascular disease (P=0.001) and myocardial infarction (P=0.016) influenced telomere attrition in the gout population (Table 2B). Additionally, in a separately performed independent linear regression model, the age at the first gout event (P=0.04), frequency of gouty flares (P=0.005) and the disease duration (P=0.035) were the most significant hallmarks of gout associating with shorter telomeres (Table 2C).
Table 2. Multiple linear regression models for telomere length measurements in patients and healthy participants.

<table>
<thead>
<tr>
<th>Number of participants</th>
<th>Standardized β</th>
<th>Standard error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gout</td>
<td>619</td>
<td>-0.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>313</td>
<td>-0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Heart failure</td>
<td>134</td>
<td>-0.22</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>270</td>
<td>-0.55</td>
<td>0.02</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>70</td>
<td>-0.40</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Standardized β</th>
<th>Standard error</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple number of flares</td>
<td>8.75 ± 12.67</td>
<td>-0.30</td>
<td>2.02</td>
</tr>
<tr>
<td>Age at the first attack</td>
<td>51.12 ± 16.75</td>
<td>-0.05</td>
<td>0.71</td>
</tr>
<tr>
<td>Disease duration</td>
<td>12.90 ± 12.28</td>
<td>-0.22</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Table 2A. Demonstrates the variables significantly associated with TL involving patients with gout and healthy individuals assessed using multiple linear regression model. Age, gender, smoking, cardiovascular disease, heart failure, stroke (non-fatal), MI (non-fatal), angina pectoris, systolic and diastolic blood pressure, BMI (kg/m²), serum UA level (mmol/L) and creatinine level (µmol/L) were included in the model as independent variables (P<0.05).

Table 2B. Includes the results of multiple linear regression analysis associating TL with cardiovascular events (heart failure, stroke (non-fatal), MI (non-fatal), angina pectoris and age only in individuals with gout (P<0.05).

Table 2C. Represents multiple linear regression analysis results where the association between TL and gout hallmarks (attack frequency, age at the first attack, disease duration, serum UA level (mmol/L), creatinine level (µmol/L)) was tested (P<0.05).

Furthermore, analysis revealed significantly shorter telomeres in patients with gout and cardiovascular events compared to gout patients without cardiovascular events (Supplementary files, table 4A). According to the linear regression model, however, there was no association between the flare frequency and cardiovascular events in patients with gout (Supplementary files, table 4B).

**Treatment might inhibit inflammation induced telomere erosion in immune-cells of gout patients**

Since the majority of gout patients were receiving anti-inflammatory and/or urate-lowering treatment, we tested if any treatment had any effect on the extend of telomeres. The treatment included allopurinol (mean 200mg/day), colchicine, corticosteroids, NSAIDs or a combination of these drugs. There was no significant difference between TL of treated (TL(±SD) = 2301 ±
2333.79) and untreated patients (TL(±SD) = 2998 ± 166.92) (N=23) (P=0.584). The insignificant
difference in TL might be due to low number of untreated patients. Since the patients were treated
with more than one drug, any drug specific effect on the extend of TL was intractable.

**Telomere shortening is not specific for certain immune cell-subsets**

To identify immune cells with a dominant effect on the complications associated with senescence,
we investigated the percentage and TL of mononuclear cell-subsets of 10 healthy participants and
10 patients with gout *(Supplementary files, table 3).*

In patients with gout, there were no significant difference in CD4⁺ T lymphocytes (24.42%), CD8⁺
Cytotoxic T lymphocytes (6.74%), CD19⁺ B lymphocytes (7.02%), CD14⁺ monocytes (7.75%), NK
cells (7.92%) and CD123⁺/CD304⁺ pDCs (0.02%) of patients with gout as compared to healthy
participants' cell-subsets that were 33.19% (P=0.11), 8.98% (P=0.061), 10.07% (P=0.157), 6.28%
(P=0.90), 7.38% (P=0.52) and 0.11% (P=0.8) respectively (Mann-Whitney U-test, P<0.05).

Moreover, CD19⁺ B lymphocytes of patients with gout had the longest telomeres and
CD123⁺/CD304⁺ pDCs the shortest. The longest and shortest telomeres in healthy participants
were also respectively in CD19⁺ B lymphocytes and CD123⁺/CD304⁺ pDCs. In direct comparison
between cell-subsets from patients with gout and healthy individuals we did not observe a
significant difference in TL.

**Telomerase activity is similar in patients with gout and healthy participants**

There was no correlation between hTERT gene expression level and TL in total PBMCs. There was
no significant difference in the mean fold change of hTERT expression level of patients with gout
(P=0.921, R²=0.013) and healthy participants (P=0.087, R²=-0.264) (Figure 3). Similarly, there
was no correlation between hTERT gene expression level and TL of the immune cell-subset
(Supplementary file, table 3).
Discussion

In this study, we show that patients with gout have shorter telomeres than healthy participants, reflecting increased cellular senescence. Telomere shortening was strongly associated with the number of gouty flares. Both gout and cardiovascular disease were independently associated with shorter telomeres. This is to our knowledge the first study to show telomere erosion in patients with gout, compared to healthy participants.

There are several strengths of this study that minimise the risk of chance observation or population bias. We exploited an independent replication cohort where DNA material was isolated and measured using a similar protocol. We observed intriguing TL differences in participants of both Dutch and New Zealand cohorts. It has been described previously that there are large differences in telomere length between Caucasians from different European descent. For instance, telomeres of inhabitants from Belgium have been shown to be six-fold longer as compared to inhabitants from Italy and are approximately twice as long as inhabitants from the United Kingdom, Ireland and Scotland[19,20]. Since the Caucasians from New Zealand have ancestors primarily from Britain and the Dutch are more closely related to the Belgians, our findings are most likely caused by an ancestral difference[20–22].

To rule out an effect of differences in telomere maintenance between patients with gout and healthy participants, we tested hTERT gene expression which has been shown to be a reliant proxy marker to measure telomerase activity[22]. We assessed hTERT gene expression in PBMCs and cell-subsets of patients with gout and healthy individuals. We did not observe any difference in hTERT gene expression level which underscores any bias due to telomerase activity as very unlikely. To minimize technical issues, we used the same protocols and same machines to measure telomere length.

To elucidate more details on underlying facets contributing to telomere attrition in gout, we quantified absolute TL in CD4+ and CD8+ T cells, CD19+B cells, CD14+ monocytes, NK cells and pDCs in patients with gout and matched healthy participants. TL of CD4+ and CD8+ T cells, CD19+
B cells, CD14+ monocytes, NK cells and pDCs showed no significant difference. In immune cell-subtypes of patients with gout, telomere shortening showed a slightly declining trend as compared to healthy participants. However, the number of included participants is insufficient to empower us to draw definite conclusions from these results. Multiple research groups have corroborated a strong link between chronic inflammation and decrease of TL in cell-subsets. For instance, studies on human immunodeficiency virus (HIV), elucidated the link between chronic infection and telomere attrition in CD4+ and CD8+ T cells[23,24]. The replicative senescence of T lymphocytes is not limited to HIV, since it is broadly investigated in association with malignancies[25], autoimmune disorders[26] and environmental mediators[27,28]. In autoimmune diseases, telomere erosion in lymphocytes has been less well delineated. Telomere erosion in CD14+ monocytes, however, was shown to be significantly associated with pro-inflammatory behaviour and, of high relevance for our findings, thereby accelerated atherosclerotic plaque formation[29]. In the elderly, diminished NK cell function was significantly associated with the extent of telomere erosion[30,31]. The literature describing telomere attrition in pDCs is rather poor and requires additional investigations. Taken together, it is known from the literature that TL of various immune cell-subsets can be impacted when chronic or recurrent inflammation is present. In our study, we could not pinpoint a single cell-subset responsible for the shortening of telomeres as observed in the PBMCs from the gout population. Since the effects on TL difference in PBMCs only came apparent in a large cohort with hundreds of patients, we believe that our lack of findings is most likely caused by a lack of power in the subset analyses. On the other hand, since we observe such similar findings between subsets, it seems to indicate that there is not one single cell-subset responsible for a large effect on TL in the whole PBMC population. The latter might point to a more generalizable effect in the cell-subset populations. Additionally, the shifting of naïve T lymphocytes towards a memory-like subset remains to be delineated, whereby the CD28 marker repertoire might be indicative of chronic inflammation-induced activation in T lymphocytes[32].
We conclude that the shorter telomeres observed in the gout population are most likely caused by increased PBMCs turnover due to on-going or frequent inflammatory episodes. This is consistent with association of reduced TL with increased flare frequency. In various tissues and cell-subtypes, accelerated cell-turnover is inversely associated with replicative senescence. In disorders involving accelerated oxidative injury and chronic immune system activation as the main manifestation, such as systemic lupus erythematosus [33], rheumatoid arthritis [32] and systemic sclerosis [34], enhanced cell-turnover and telomere erosion has been extensively described. Furthermore, we observed no significant TL difference in treated patients with gout as compared to patients without treatment. In addition, patients with the most frequent flares had the shortest telomeres. This might indirectly imply a modulating effect of inflammation on telomere length and thereby replicative senescence.

The results of our study underline the strong association between inflammation, senescence and cardiovascular disease, as suggested previously [35]. Recently, it has become apparent that rheumatic diseases confer risk of cardiovascular disease development. In rheumatoid arthritis, this risk approaches the risk of patients with diabetes [36]. The pathogenesis of this increased risk is largely enigmatic, but is proposed to arise from a chronic inflammatory environment, causing vascular damage and plaque formation [37,38]. The occurrence of atherosclerotic plaques has been related to an increasing chronological age [11]. On the other hand, there is a large role for biological ageing as well which has been demonstrated by the influx of pro-inflammatory senescent immune cells in the plaque contributing to plaque instability and rupture and eventually cardiovascular and cerebrovascular events [39]. The connection between replicative senescence and the presence of cardiovascular disease has been described before in the literature [40]. Likewise, the prevalence of myocardial infarction in the elderly with shorter leukocytes' TL is about 3 times higher [41,42,40]. A recent systemic review confirmed this association by combining the telomere data and vascular morbidity of over 50,000 individuals [10].
In summary, we present decreased TL as a feature of gout and confirm TL as an independent factor associated with flare frequency and cardiovascular disease. These data suggest that intensive treatment of gout may prevent replicative senescence and subsequently reduce the associated cardiovascular risk. However large prospective studies are needed to test this possibility.
ACKNOWLEDGEMENTS AND FUNDING INFORMATION

This study was partly funded by the NWO VIDI grant of T.R.D.J. Radstake. J.C.B. is supported by a VENI Award from the Netherlands Organization for Scientific Research (N.W.O. project number 91614041). The New Zealand portion of the study was funded by the Health Research Council of New Zealand, New Zealand Lottery Health, Arthritis New Zealand and the University of Otago, New Zealand.
ETHICAL APPROVAL INFORMATION

This study was performed according to the guidelines of the Declaration of Helsinki and meets the ethical approval of the Ethical and Review committee of the University Medical Centre of Utrecht, Rijnstate hospital in Arnhem (both Netherlands) and the Multi-Region Ethics Committee in New Zealand. All the participants in this study gave their informed consent to participate.
COMPETING INTERESTS

Non declared.
AUTHORSHIP

N.V. contributed to research design, performed experiments, analyzed data and contributed to writing the paper; L.B.E.K., E.V.L., N.D., L.K.S., T.R.M., M.J., contributed to patients’ recruitment, interpreted data, and contributed to writing the manuscript; C.W. performed experiments and contributed to data analysis; M.R., P.G.S., interpreted data, and contributed to writing the manuscript; J.C.A.B. and T.R.D.J.R designed research, interpreted data and contributed to writing the manuscript.

AUTHOR CONTRIBUTION

All authors approved the final version after being involved in drafting and revising the article for important intellectual content. As the corresponding author, Dr. Broen had full access to the data and takes responsibility for the accuracy of the performed analysis and the integrity of the data.
LICENCE FOR PUBLICATION

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DATA SHARING STATEMENT

Other than the included data, there is no additional data available regarding this manuscript.
Reference


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Healthy participants
\[ Y = -0.006349 \times X + 3.99 \]
\[ P < 0.0001 \]

Gout
\[ Y = -0.006084 \times X + 3.79 \]
\[ P = 0.0010 \]
Healthy participants
$Y = -0.01089X + 3.94$
$P < 0.0001$

Gout
$Y = -0.004926X + 3.51$
$P = 0.0014$