Effectiveness of etanercept vs cyclophosphamide as treatment for patients with amyloid A amyloidosis secondary to rheumatoid arthritis

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Abstract

Objectives. To compare the effectiveness of an alkylating agent with that of a biologic agent in the treatment of patients with amyloid A (AA) amyloidosis secondary to RA and to assess the association of the serum AA (SAA) 1.3 allele with treatment.

Methods. CYC and etanercept (ETN) were administered to 62 and 24 RA patients, respectively, who were confirmed with biopsy as having AA amyloidosis. We evaluated whether the SAA1.3 allele, a factor indicating genetic risk and poor prognosis of Japanese RA patients with AA amyloidosis, influenced treatments and retrospectively analysed the effectiveness of both agents via statistical methods.

Results. Two treatment groups were similar, except for the SAA1.3 genotype ($P=0.015$) and duration of AA amyloidosis since diagnosis ($P<0.001$). Also, patients given ETN had somewhat worse renal function, i.e. 24-h proteinuria ($P=0.02$), at the initiation of treatment. ETN demonstrated greater effectiveness than CYC, as shown by significantly improved levels of serum CRP and serum albumin (both $P<0.01$) and estimated glomerular filtration rate (eGFR; $P=0.032$). ETN improved survival ($P=0.025$), and the hazard ratios for the risk of death endpoint with eGFR and 24-h proteinuria were significant by $P=0.024$ and $P=0.025$, respectively. The SAA1.3 allele did not affect the response to medications in AA amyloidosis secondary to RA.

Conclusion. ETN treatment was more effective than CYC treatment, and CRP, albumin and eGFR may be valuable biomarkers for analysis. The SAA1.3 allele was not a factor affecting treatment in Japanese patients with AA amyloidosis secondary to RA.

Key words: AA amyloidosis, CYC, etanercept, RA, SAA1.3 allele, biomarkers, eGFR.

Introduction

With new biologic therapies, both treatment and understanding of the roles of cytokines in RA have seen considerable progress. Biologics are recommended for patients with RA who have a suboptimal response or intolerance to traditional DMARDs, such as MTX. RA is a common type of chronic inflammatory arthritis, and among extra-articular complications in RA, amyloid A (AA) amyloidosis is a serious, potentially life-threatening disorder caused by deposition in organs of AA amyloid fibrils, which derive from the circulatory acute-phase reactant, serum AA protein (SAA) [1]. Early diagnosis and rapid subsequent treatment are essential because patients with advanced disease cannot usually undergo intensive therapy. Specific treatment of AA amyloidosis caused by RA aims to stop SAA production. Cytotoxics such as chlorambucil and CYC and biologics such as anti-TNF-α inhibitors and anti-IL-6 receptor antibody are reportedly useful for both RA and AA amyloidosis [2, 3]. We previously showed that the genetic predisposition allele SAA1.3 was not only a univariate predictor of survival but also a risk factor for association of AA amyloidosis with RA in Japanese patients [4]. In view of our earlier
reports on the efficacy of etanercept (ETN) [5] and CYC [6] given alone for AA amyloidosis secondary to RA, we compared the effectiveness of ETN and CYC, and we assessed biomarkers and analysed the effect of the SAA1.3 allele on these treatments.

**Patients and methods**

**Patients**

This retrospective cohort study compared the effectiveness of CYC and ETN for RA patients with AA amyloidosis who were homozygous for the SAA1.3 allele or other polymorphisms. All RA patients fulfilled the 1987 ACR criteria for RA [7]. Sixty-two RA patients received CYC and 24 received ETN (Table 1); all had biopsy-confirmed AA amyloidosis. We reviewed the clinical records of enrolled patients who were followed from January 1995 to December 2010 at our centre. When patients with RA showed clinical features indicating possible AA amyloidosis, such as proteinuria, thyroid dysfunction, weight loss, repeated constipation and diarrhoea, routine screening including tissue biopsy and an upper gastrointestinal serial examination was undertaken, with patients giving informed consent. The presence of AA amyloid deposits was confirmed histologically via positive Congo Red staining, potassium permanganate susceptibility and green birefringence seen by polarization microscopy after Congo Red staining, as well as immunohistochemical analysis using anti-AA antibody and anti-immunoglobulin light-chain antibody to differentiate anti-immunoglobulin light-chain amyloidosis. We excluded patients whose clinical AA amyloidosis diagnoses were not confirmed by biopsy. The Ethical Committee of the Kumamoto Center for Arthritis and Rheumatology permitted our rheumatology group to perform the serial retrospective studies. All patients gave consent for the tissue biopsy and the study.

Patients with RA had been treated with NSAIDs, prednisolone (PSL), at least one DMARD (i.m. and oral gold preparations, lobenzarit disodium, D-Pen, bucillamine, SSZ or actarit) and immunosuppressive agents (AZA, tacrolimus, LEF or MTX), but were often refractory to these agents. Although CYC was not licensed for use as RA treatment by the Japanese health insurance system before February 2011, we used CYC for RA patients under informed consent and investigated its efficacy from December 2004.

**Determination of SAA1 genotype**

We performed PCR-based restriction fragment length polymorphism (RFLP) analysis, as described elsewhere [8].

**Demographic and clinical variables**

Age, sex and duration of RA and AA amyloidosis were recorded, as were changes in laboratory indices. Clinical evaluations of disease activity included CRP, SAA, ESR, RF, serum albumin (Alb), serum creatinine (Crea), 24-h proteinuria and estimated glomerular filtration rate (eGFR). Use of DMARDs, immunosuppressants or PSL from the time of RA onset to the index time was noted.

We chose CRP as an indicator of rheumatoid inflammation and Alb as an indicator of severity of AA amyloidosis [9]. Because renal dysfunction is the most common symptom in AA amyloidosis secondary to RA, we selected Crea and eGFR to assess treatment effectiveness. We calculated eGFRs via the nomogram for modification of diet reported in a Japanese renal disease study (index 0.741) using Crea measured by using an enzymatic method [10]. We also obtained information on drugs including DMARDs, NSAIDs, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers. We recorded clinical symptoms and arthritis activity for each time point. We carefully checked for adverse effects of immunosuppressants, e.g. infection risks, myelosuppression, haemorrhagic cystitis and carcinogenesis.

**Treatment**

CYC was administered until December 2004 at a dose determined by the level of 24-h creatinine clearance (Ccr) as reported previously [6]. ETN was given as 25 mg s.c. injections twice weekly until December 2010. We recorded known risk factors for chronic kidney disease, including the use of NSAIDs, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers or other comorbidities (hypertension, dyslipidaemia and diabetes mellitus). We monitored the effects of these drugs or diseases on renal function to differentiate renal deterioration arising from drugs or comorbidities from that caused by AA amyloidosis.

**Assessment**

We monitored biomarker levels and compared initial (before treatment) and last (after treatment) values. We used statistical analysis to assess the effects of the SAA1.3 allele on therapies.

**Statistical analysis**

We determined the onset of RA by reviewing charts after AA amyloidosis diagnosis had been confirmed. Clinical symptoms at presentation were the main reason for physicians to obtain tissue biopsies to demonstrate AA amyloid deposits. We estimated survival curves via the Kaplan–Meier technique; we analysed statistical differences between two curves by the log-rank test. We used Cox proportional hazards models to assess the effects of treatments on eGFR and 24-h proteinuria, with risk of death as the endpoint. We used two-way repeated-measures analysis of variance to simultaneously estimate the effects of SAA1.3 or treatments on individual changes in biomarkers. In this model, individual change was defined as within-subject factors; categorical groups, i.e. polymorphisms of the SAA1.3 allele, and treatments were defined as between-subject factors. To determine the factor affecting individual change, a combined factor (within and between) was defined as interaction. We evaluated the significant effects of these factors via analysis of variance. We determined significant interaction of the effects of groups (SAA1.3 or treatments) on changes in individual markers. Findings were statistically significant at
We used SPSS Statistics 17.0, Base and Advanced, (SPSS Inc., Chicago, IL, USA) for statistical analyses.

**Results**

**Patients’ background**

Table 1 provides patients’ clinical characteristics and laboratory findings. Despite treatments being administered during different periods, clinical and laboratory findings of both groups were quite similar at the start of each treatment, except for the SAA1.3 genotype and duration of AA amyloidosis since diagnosis ($P = 0.015$ and $P < 0.001$, respectively). With regard to biomarkers indicating renal dysfunction, ETN caused worse kidney damage than did CYC. During the study, patients died in each treatment group at almost the same rate. In the CYC group, congestive heart failure and infectious pneumonia occurred frequently. Patients given ETN had a lower rate of congestive heart failure as cause of death, suggesting an inhibitory effect on progressive heart failure.

**Effect of treatments on biomarkers**

Years since RA onset and years since diagnosis of AA amyloidosis were significantly different for SAA1.3 homozygosity vs other genotypes ($15.6 ± 7.8$ vs $21.4 ± 9.9$, $P = 0.046$ and $7.44 ± 4.9$ vs $9.7 ± 4.5$, $P = 0.016$, respectively). Comparison of CRP, Alb, eGFR and Crea for both groups at initial and final observations, disregarding SAA1.3 allele polymorphisms, showed that ETN reduced serum CRP levels and increased serum Alb levels more than did CYC (ETN vs CYC, CRP: from $4.7 ± 0.8$ to $0.5 ± 0.3$ mg/dl vs from $4.0 ± 1.6$ to $2.8 ± 1.2$ mg/dl, $P < 0.01$; Alb: from $2.6 ± 0.4$ to $3.5 ± 0.4$ g/dl vs from $2.8 ± 0.3$ to $2.8 ± 0.5$ g/dl,

### TABLE 1 Clinical characteristics and laboratory findings for patients

<table>
<thead>
<tr>
<th></th>
<th>CYC (n = 62)$^b$</th>
<th>ETN (n = 24)$^c$</th>
<th>$P$-value$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female, n</td>
<td>12/50</td>
<td>4/20</td>
<td>1.000</td>
</tr>
<tr>
<td>SAA1.3 allele, homozygous/others, n</td>
<td>22/40</td>
<td>16/8</td>
<td>0.015</td>
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<tr>
<td>Months since RA onset</td>
<td>176.0 (111.0)</td>
<td>195.3 (88.3)</td>
<td>0.447</td>
</tr>
<tr>
<td>Months since diagnosis of AA amyloidosis</td>
<td>22.9 (41.7)</td>
<td>67.5 (42.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Months of treatment</td>
<td>38.0 (27.4)</td>
<td>34.0 (23.1)</td>
<td>0.526</td>
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<tr>
<td>Steinbrocker’s classification$^e$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stage: II/III/IV, n</td>
<td>5/18/39</td>
<td>3/7/14</td>
<td></td>
</tr>
<tr>
<td>Class: 2/3/4, n</td>
<td>38/18/6</td>
<td>14/7/3</td>
<td></td>
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<tr>
<td>MTX therapy, yes/no, n</td>
<td>31/31</td>
<td>15/9</td>
<td>0.297</td>
</tr>
<tr>
<td>PSL dosage, mg/day</td>
<td>9.91 (5.88)</td>
<td>7.97 (4.96)</td>
<td>0.156</td>
</tr>
<tr>
<td>CRP, mg/dl</td>
<td>3.99 (1.72)</td>
<td>3.89 (1.97)</td>
<td>0.820</td>
</tr>
<tr>
<td>SAA, µg/ml</td>
<td>294.8 (166.0)</td>
<td>327.0 (223.4)</td>
<td>0.467</td>
</tr>
<tr>
<td>eGFR, ml/min/1.73 m$^2$</td>
<td>29.2 (23.9)</td>
<td>31.2 (20.6)</td>
<td>0.714</td>
</tr>
<tr>
<td>Crea, mg/dl</td>
<td>2.04 (0.95)</td>
<td>2.23 (1.27)</td>
<td>0.465</td>
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<tr>
<td>24-h urinary protein, g</td>
<td>1.53 (0.84)</td>
<td>2.09 (1.27)</td>
<td>0.020</td>
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<tr>
<td>Alb, g/dl</td>
<td>2.82 (0.32)</td>
<td>3.04 (0.68)</td>
<td>0.128</td>
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<tr>
<td>RF, U/ml</td>
<td>237.8 (262.1)</td>
<td>200.8 (154.3)</td>
<td>0.519</td>
</tr>
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<td>Cause of death, n (%)</td>
<td>26 (41.9)</td>
<td>8 (33.3)</td>
<td>0.464</td>
</tr>
<tr>
<td>Infectious pneumonia</td>
<td>6</td>
<td>3</td>
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<tr>
<td>Sepsis</td>
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<td></td>
</tr>
<tr>
<td>GI ulcer/bleeding</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>Interstitial pneumonia</td>
<td>4</td>
<td>1</td>
<td></td>
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<tr>
<td>Myocardial infarction</td>
<td>2</td>
<td></td>
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<td>Congestive heart failure</td>
<td>7</td>
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<tr>
<td>Renal failure</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
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<td></td>
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</tr>
</tbody>
</table>

$^a$Baseline data were obtained at the initiation of each treatment. All patients, except those who died, were followed from January 1995 to December 2010. The last observations were made in December 2004 for the CYC group and December 2010 for the ETN group. Values are represented as mean (s.d.) unless otherwise noted. Causes of death of patients who died during the study are shown, and these causes were most closely related to their death. $^b$CYC was given until December 2004. Thirty-six alive [8 males, 28 females; mean (s.d.) age 65.7 (10.8) years] and 26 dead [3 males, 23 females; mean (s.d.) age 71.9 (8.5) years]. CYC was administered according to the level of 24-h Ccr; Ccr $\geq$ 80 (ml/min): 100 (mg/day), Ccr $< 80$ (ml/min): 75 (mg/day), Ccr $< 60$ (ml/min): 50 (mg/day), Ccr $< 40$ (ml/min): 25 (mg/day), Ccr $< 20$ (ml/min): 10 (mg/day). $^c$ETN was given until December 2010. Sixteen alive [3 males, 13 females; mean (s.d.) age 64.2 (8.5) years] and 8 dead [1 male, 7 females; mean (s.d.) age 66.0 (7.8) years]. $^d$Student’s t-test was performed to compare CYC and ETN, with $P < 0.05$ indicating a significant result. $^e$Steinbrocker’s classification [11]. GI: gastrointestinal.

$P < 0.05$. We used SPSS Statistics 17.0, Base and Advanced, (SPSS Inc., Chicago, IL, USA) for statistical analyses.
Thus ETN significantly improved serum CRP and Alb levels and was clearly more effective than CYC. CRP and Alb interactions with polymorphism (homozygous for SAA1.3 or other polymorphisms) showed no significance \((P=0.777~\text{and}~P=0.715,\text{respectively})\), but CRP and Alb interactions with treatment (ETN or CYC) demonstrated significant results (both \(P<0.01\)). Within-subject analysis showed that treatments improved eGFR: ETN from \(21.8\pm18.9\) to \(24.9\pm18.7\frac{\text{ml/min}}{1.73\text{m}^2}\) vs CYC from \(29.3\pm12.7\) to \(18.6\pm9.3\frac{\text{ml/min}}{1.73\text{m}^2}\), \(P=0.035\), with ETN’s effect on eGFR being significant \((P=0.032)\). ETN increased eGFR, thus improving the decreased renal function caused by AA amyloidosis, more than did CYC (Fig. 1A), but this effect was not related to SAA1.3 allele polymorphisms (Fig. 1B). Neither treatment affected Crea levels.

### Relationship between SAA1.3 genotype and treatment

SAA1.3 did not affect treatment in both groups of patients, as shown by interactions of SAA1.3 with CRP, Alb, eGFR and Crea \((P=0.777, P=0.715, P=0.465\text{~and}~P=0.228,\text{respectively})\).

### Survival curves of SAA1.3 genotype and treatment

Because ETN was more effective than CYC, according to CRP, Alb and eGFR measures, we calculated the hazard ratio between ETN and CYC as a survival parameter. According to Cox proportional hazards survival analysis, ETN significantly improved survival \((P=0.025)\). Also, the Kaplan–Meier curves showed a significant difference between ETN and CYC (Fig. 1C). The hazard ratio for ETN showed significant results for the risk of death endpoint (eGFR: \(P=0.024\) and 24-h proteinuria: \(P=0.025,\text{respectively}\) but CYC did not (Table 2).

### Discussion

The goal of AA amyloidosis therapy is to control the underlying disorder. Treatment suppressing inflammatory activity reduces circulatory levels of SAA, an acute-phase reactant. In AA amyloidosis secondary to RA, treatment focused on using cytotoxic drugs such as CYC and

![Fig. 1](https://example.com/figure1.png)

**Fig. 1** Relationship between therapies (ETN and CYC treatments) and eGFR, SAA1.3 allele genotype and survival.

(A) Changes in eGFR between initial and last visits as a function of treatment. (B) Changes in eGFR between initial and last visits as a function of SAA 1.3 allele genotype (homozygosity or other polymorphisms). (C) Kaplan–Meier survival curves after treatment with ETN (continuous line) and CYC (dotted line; \(P=0.025\), log-rank test).
Hazard ratio for each treatment using the Cox proportional hazards models to assess the effects of treatments on the risk of death as the endpoint, being determined by two variables, eGFR and 24-h proteinuria.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
<th>Hazard ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETN</td>
<td>0.949 (0.907, 0.993)</td>
<td>0.024</td>
<td>1.779 (1.074, 2.946)</td>
<td>0.025</td>
</tr>
<tr>
<td>CYC</td>
<td>0.951 (0.894, 1.012)</td>
<td>0.110</td>
<td>1.161 (0.542, 2.488)</td>
<td>0.701</td>
</tr>
</tbody>
</table>

Hazard ratio for each treatment

chlorambucil [12, 13] and more recently on TNF-α inhibitors and IL-6 receptor antibody [2, 14]. Before the advent of biologics, encouraging reports of alkylation agents benefiting RA patients with AA amyloidosis were published. The rationale of this treatment seems to be similar to autologous stem cell transplantation, which generates new self-tolerant lymphocytes after alkylation agent treatment by eliminating self-reactive lymphocytes [15].

In the light of the reported superiority of CYC compared with MTX for managing RA patients with AA amyloidosis [4], using alkylation agents may improve AA amyloidosis. Cytotoxic drugs and cytokine inhibitors affect AA amyloid deposits by suppressing SAA production. Also, anti-TNF-α therapies, by inhibiting the expression of receptors of advanced glycation end-products, may reduce interactions between AA amyloid fibrils and receptors of advanced glycation end-products and thereby prevent AA-mediated cell toxicity [16, 17]. Thus our finding that ETN had greater effects on AA amyloidosis secondary to RA than CYC did was not unexpected, and early therapeutic intervention in RA may avoid the complication of AA amyloidosis by controlling rheumatoid disease activity [18].

In Japan, the use of MTX to treat patients with RA was permitted in 1999, the maximum dose being 8 mg/week until February 2011; use of ETN was allowed in 2005. In our study, the time from diagnosis of AA amyloidosis was shorter for the CYC group than the ETN group (Table 1), but treatment strategies and DMARDs used were the same, except for the use of biologics. Although MTX is now considered an anchor drug for RA treatment, it was used infrequently for AA amyloidosis patients because of the renal damage it can cause. No significant differences between groups in MTX therapy were found (Table 1).

The recovery of Alb biosynthesis, improved acute-phase response and ameliorated eGFR are all demonstrable endpoints, and we suspect that Alb reflects the severity of AA amyloidosis [9, 19]. We found that the different therapies rather than SAA1.3 allele polymorphism influenced changes in CRP and Alb. Also, eGFR may reflect diminished renal blood flow, and only ETN improved eGFR, thus indicating better renal function and greater efficacy of ETN than CYC (Fig. 1A). We found no evidence linking SAA1.3 allele to treatment efficacy (Fig. 1B).

The present study does have certain limitations such as small sample size, a retrospective comparative investigation performed at one centre, and CYC and ETN being administered during different time periods. However, our study determined that SAA1.3 polymorphisms had no effect on treatment and ETN had greater effects than CYC on AA amyloidosis secondary to RA. The rationale for using biologics in AA amyloidosis derives from their ability to reduce levels of serum pro-inflammatory cytokines, which regulate SAA synthesis [20]. ETN can be used for RA patients with AA amyloidosis, even in those undergoing dialysis [21]. Because RA patients with AA amyloidosis carrying the SAA1.3 allele are likely to have a poor prognosis and outcome [4], careful follow-up of these RA patients is desirable to diagnose AA amyloidosis as early as possible.

**Rheumatology key messages**

- ETN is more effective than CYC for the treatment of AA amyloidosis secondary to RA.
- Genetic predisposition SAA1.3 allele is not associated with therapies for AA amyloidosis secondary to RA.

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**Disclosure statement:** The authors have declared no conflicts of interest.

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