

Baseline Numbers of Circulating CD28-negative T Cells May Predict Clinical Response to Abatacept in Patients with Rheumatoid Arthritis

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ABSTRACT. *Objective.* To evaluate the number of circulating CD28-negative (CD28⁻) T cells as a predictor of clinical response to abatacept in patients with rheumatoid arthritis (RA).

Methods. Peripheral blood CD28⁻ T cell subsets were evaluated by flow cytometry at baseline in 32 patients with RA treated with abatacept. Receiver-operator curves were applied to examine the predictive value of T cell populations and to choose the cutoff for the best performance of the test. Remission was defined using the Disease Activity Score 28 based on C-reactive protein.

Results. The overall predictive values of the CD8+CD28⁻ and CD4+CD28⁻ cells for remission after 6 months of abatacept therapy were 0.802 (SE 0.078) and 0.743 (SE 0.089), respectively. Cutoff values of < 87 CD8+CD28⁻ cells/ μ l and < 28 CD4+CD28⁻ cells/ μ l had 80.0% sensitivity and 81.8% specificity (Fisher test: $p = 0.001$), and 60.0% sensitivity and 77.3% specificity ($p = 0.043$), respectively, for prediction of remission at 6 months. Patients having low baseline numbers of CD8+CD28⁻ T cells had a more than 4-fold higher probability of achieving remission within 6 months than patients with higher levels of these cells.

Conclusion. A simple laboratory measure, the baseline number of circulating CD28⁻ T cells, predicted remission after 6 months of abatacept treatment in patients with RA. (J Rheumatol First Release Aug 1 2011; doi:10.3899/jrheum.110386)

Key Indexing Terms:

ABATACEPT
PREDICTIVE FACTORS

RHEUMATOID ARTHRITIS

CD28-NEGATIVE T CELLS
REMISSION

A wide range of biological agents targeted at specific components of immune response are available for treatment of rheumatoid arthritis (RA). There is much interest in the definition of predictors of response to different agents, with the aim of development of personalized therapy¹. However, no such data are currently available for predicting response to abatacept¹, a fusion protein (CTLA4Ig) that can act as a T cell costimulation blocker, binding to CD80 and CD86 on antigen-presenting cells, and thereby preventing the engagement of CD28 on T cells².

We evaluated the number of circulating CD28-negative (CD28⁻) T cells as a predictor of clinical response to abatacept in patients with RA. This measure was chosen because many studies have demonstrated that in patients with RA (as in many other clinical conditions characterized by immune activation) there is an increased number of peripheral blood CD28⁻ T cells^{3,4,5,6}. Since downmodulation of CD28 expression can be achieved *in vitro* through prolonged stimulation with specific peptide antigens⁷, or with cytokines like tumor

necrosis factor- α (TNF- α)⁸, it can be hypothesized that the number of circulating CD28⁻ T cells might represent a measure of the burden of prolonged previous immune stimulation. Moreover, downmodulation of CD28 expression can also be achieved *in vitro* through engagement with its ligands (CD80/CD86)⁹, and since this engagement is blocked by abatacept, we hypothesized that abatacept also prevents generation of the CD28⁻ population. Indeed, we have shown that the number of CD28⁻ T cells is reduced after therapy with abatacept⁴, and that such a decrease is correlated with clinical response. Therefore, we investigated a relationship between the number of baseline circulating CD28⁻ T cells and the clinical efficacy of abatacept.

MATERIALS AND METHODS

Patients. Thirty-two consecutive patients with a diagnosis of RA, as defined by the 1987 American College of Rheumatology criteria¹⁰, who previously had failed one or more TNF-blocking therapies were treated with abatacept by intravenous infusion according to baseline weight (< 60 kg received 500 mg, 60–100 kg inclusive received 750 mg, and > 100 kg received 1000 mg) on Days 1, 15, and 29 and then monthly. Sixteen of these patients took part in a previous study on T cell populations⁴. The study was conducted in accord with the recommendations of the Helsinki Declaration, and was approved by the local ethical committee. Clinical assessment of patients was performed using the Disease Activity Score 28 based on C-reactive protein (DAS28-CRP) and clinical response was evaluated with the European League Against Rheumatism (EULAR) response criteria¹¹.

Leukocyte phenotype analysis. Peripheral blood lymphocyte surface markers

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were evaluated by 4-color flow cytometry (Cytomics FC-500, Beckman Coulter Inc., Fullerton, CA, USA) at the time of the first infusion with abatacept, using monoclonal antibodies (CD3, CD4, CD8, CD28; Beckman Coulter), to identify lymphocyte subpopulations. Absolute cell count was determined by single-platform analysis using Flow-Count beads (Beckman Coulter).

Statistical analysis. Data were expressed as the median (10th–90th percentile). Comparisons were made using the Mann-Whitney and Fisher's exact test or chi-square test, as appropriate.

Receiver-operator curves (ROC) were applied to examine the predictive value of T cell populations and to choose the cutoff for the best performance of the test. The overall diagnostic accuracy of the test was estimated using the area under the ROC curve¹². Patients were classified into 2 groups according to cutoff values derived from the ROC curve. The log-rank test was applied to compare the times to good clinical response and remission, and the relative risks (RR) of clinical responses in the 2 groups were calculated.

RESULTS

Thirty-two consecutive patients with RA treated with abatacept at our institution were included in the study. Clinical data (Table 1) demonstrate that this was a cohort with active disease (median DAS28-CRP 5.7) and patients were heavily pretreated (median number of nonbiological disease-modifying antirheumatic drugs: 4, and TNF-blocking

agents: 2; 8 patients had also previously been treated with rituximab).

As shown in Figure 1, remission (DAS28-CRP < 2.6 for at least 2 consecutive visits) was achieved in 10 (31%) patients after 6 months of therapy, in 13 (41%) patients after 12 months, and in 16 (50%) patients considering any timepoint during abatacept therapy. A EULAR good clinical response was obtained in 15 (47%), 17 (53%), and 20 (62%) patients after 6 months, 12 months, and at any time during abatacept therapy, respectively.

As shown in Table 1, there was no significant difference in demographic and clinical features at baseline between patients who achieved clinical remission within 6 months and those who did not, except that those achieving remission were not previously treated with rituximab ($p = 0.03$). In these patients the number who discontinued TNF-blocking agents for adverse events and not for inefficacy was higher, with borderline statistical significance ($p = 0.053$). Baseline circulating CD8+CD28– and CD4+CD28– T cell numbers were significantly lower in patients who achieved clinical remission at 6 months than in other patients ($p = 0.007$, $p = 0.03$, respectively). In a similar way, patients who obtained good clinical

Table 1. Main demographic and clinical characteristics of 32 with rheumatoid arthritis patients-treated with abatacept. Data are expressed as median (10th–90th percentile).

	All Patients, n = 32	Evaluation of Remission at 6 Months		p
		Remission, n = 10	No Remission, n = 22	
Sex, M/F	3/29	1/9	2/20	0.93
Age, yrs	56 (39–71)	56.5 (47–67)	55.5 (39–71)	0.93
Disease duration, yrs	9 (3–21)	8.5 (4–20)	10.5 (3–21)	0.41
Median no. comorbidities	1 (0–3)	1 (0–3)	1 (0–2)	0.14
Ever smokers	10/32	2/10	8/22	0.35
Rheumatoid factor	25/32	8/10	17/22	0.86
Anti-CCP	23/28	8/8	15/20	0.12
No. previous DMARD	4 (2–6)	3 (2–5)	4 (2–7)	0.08
Health Assessment Questionnaire (HAQ)	1.325 (0.75–1.975)	1.19 (0.71–2.01)	1.44 (0.87–1.87)	0.46
No. previous TNF- α blocking agents (TNFBA)	2 (1–3)	2 (1–3)	2 (1–3)	0.89
Cause of TNFBA suspension				
Inefficacy	26	6	19	0.09
Adverse events	6	4	2	0.053
Previous rituximab (inefficacy)	8/32	0/10	8/22	0.03
DAS28 (CRP) at baseline	5.70 (4.17–6.88)	5.70 (4.28–6.94)	5.71 (3.91–6.36)	0.31
Median dose of MTX at baseline, mg/week	10 (0–15)	10 (0–15)	11.25 (0–15)	0.93
Median dose of prednisone at baseline, mg/day	5 (3.6–10)	5 (3.6–10)	5 (3.6–9.1)	0.59
Suspension of abatacept (within 6 mo)	6	0	6	0.08
Inefficacy	5		5	
Side effects	0		0	
Lack of compliance	1		1	
Serious adverse events during abatacept therapy	1 (stroke)	0	1	0.49
No. circulating CD4+CD27– T cells at baseline (cells/microliter)	36 (11–150)*	26 (6–52)	59 (13–245)	0.03
No. circulating CD8+CD28– T cells at baseline (cells/microliter)	111 (38–261)*	53 (23–121)	132 (44–290)	0.007

* Normal values (25th–75th percentile) among 27 healthy volunteers (median age 49 yrs): 14–35 CD4+CD28– cells/microliter; 54–129 CD8+CD28– cells/microliter. Anti-CCP: anticyclic citrullinated peptide antibodies; DMARD: disease-modifying antirheumatic drug; TNF: tumor necrosis factor; DAS28: 28-joint Disease Activity Score; CRP: C-reactive protein; MTX: methotrexate.

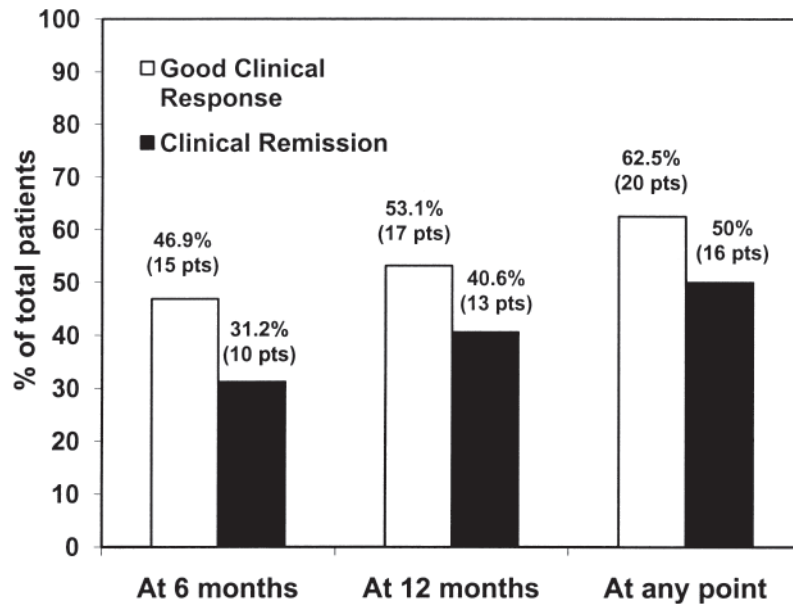


Figure 1. Proportions of individuals achieving European League Against Rheumatism good clinical response or remission (28-joint Disease Activity Score-C-reactive protein < 2.6) among 32 patients with rheumatoid arthritis treated with abatacept.

response within 6 months of therapy differed from those who did not only for the lower number of patients who discontinued TNF-blocking agents because of inefficacy (9/15 vs 17/17; $p = 0.015$), and for the lower number of circulating CD4+CD28- T cells at baseline [31/ μl (10th–90th percentile: 9–69) vs 75/ μl (13–264); $p = 0.047$], whereas the number of circulating CD8+CD28- T cells was not significantly different ($p = 0.108$; other data not shown).

ROC curves (Figure 2) illustrate the incidence of remission and good clinical response at 6 months predicted by numbers of CD28- T cells at baseline. The overall predictive values of the CD8+CD28- and CD4+CD28- cells for remission were 0.802 (SE 0.078) and 0.743 (SE 0.089), respectively.

Cutoff values were derived from the ROC curve and they were < 87 CD8+CD28- cells/ μl and < 28 CD4+CD28- cells/ μl . They had 80.0% sensitivity and 81.8% specificity (Fisher test: $p = 0.001$) and 60.0% sensitivity and 77.3% specificity ($p = 0.043$), respectively, for prediction of clinical remission at 6 months.

Predictions for good clinical response at 6 months were less efficient: the area under the ROC curve was 0.669 (SE 0.095) for CD8+CD28- cells and 0.706 (SE 0.092) for CD4+CD28- cells. Nevertheless, significant cutoff values were derived at < 146 cells/ μl ($p = 0.04$) and < 60 cells/ μl ($p = 0.02$), respectively.

Kaplan-Meier cumulative achievements of remission and good clinical response in groups of patients classified according to the numbers of baseline CD28- T cells are shown in Figure 3.

The calculated relative risks associated with the remission

at 6 months were 4.4 (95% CI 1.72–11.25, $p = 0.0008$) for < 87 CD8+CD28- cells/ μl ($p = 0.0001$) and 3.3 (95% CI 1.19–9.16, $p = 0.018$) for < 28 CD4+CD28- cells/ μl .

Prediction of CD8+CD28- T cell number was less significant than at 6 months for remission at 12 months [RR 2.67 (95% CI 1.13–6.29, $p = 0.02$)] or at any point in therapy [RR 2.78 (95% CI 1.36–5.68, $p = 0.004$)]. On the other hand, neither the predictivity of CD8+CD28- T cells for good clinical response, nor that of CD4+CD28- T cells for remission or good clinical response, at 12 months or at any time was significant (data not shown).

As described⁴, the number of CD28- T cells decreased progressively during therapy with abatacept. Data at first times of clinical remission and good clinical response are reported here. Six patients achieved remission more than 6 months after starting abatacept therapy. The circulating numbers of CD28- T cells at the time of remission in these patients were not significantly different from those observed in the 10 patients who achieved remission within 6 months (Figure 4). Analogously, 5 patients obtained a good clinical response more than 6 months after the start of therapy, and they were not different, comparing CD28- T cell numbers at the time of response, from 15 patients obtaining such a response in the first 6 months (Figure 4). These data suggest that in both groups clinical response was related to a low number of circulating CD28- T cells ($p < 0.01$ vs baseline values for all the comparisons).

DISCUSSION

In our experience, a simple laboratory test, based on flow

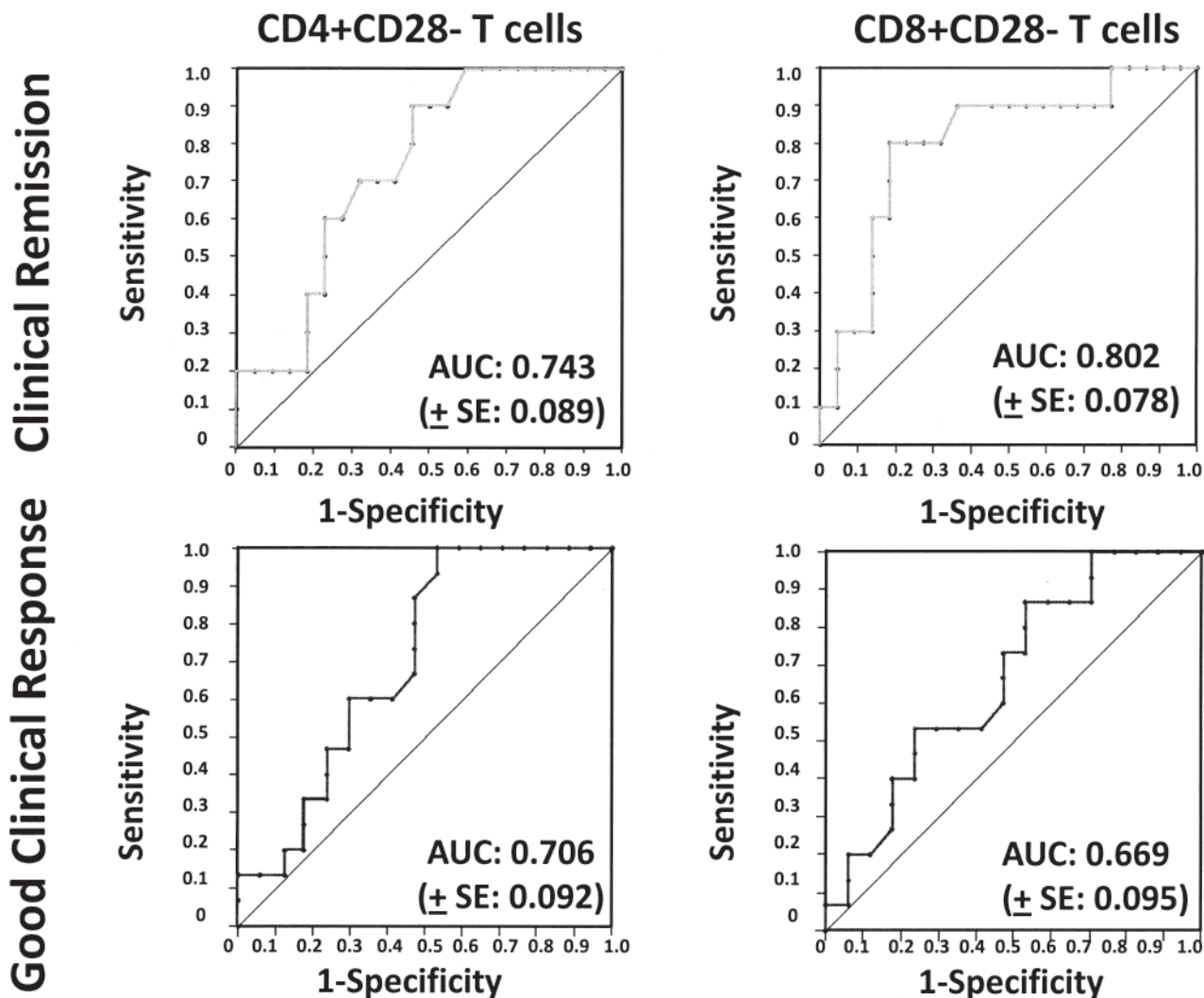


Figure 2. Receiver-operator curves for numbers of baseline CD4+ or CD8+ CD28- T cells for prediction of EULAR good clinical response or remission (DAS28-CRP < 2.6) at 6 months among 32 patients with RA treated with abatacept. AUC: area under the curve.

cytometry evaluation of numbers of circulating CD28- T cells, significantly predicted the clinical response to 6 months of therapy with abatacept in patients with RA. This test is rapid, widely available, and relatively inexpensive. The main result of our study was that patients having baseline numbers of circulating CD8+CD28- T cells below 87 cells/ μ l (n = 10) had a more than 4-fold higher probability of achieving DAS28-CRP remission within 6 months than patients with higher levels of these cells (n = 22). Patients achieving remission did not differ from other patients in demographic and clinical variables other than numbers of CD28- T cells. The only exception was that they possibly had a disease less resistant to other biological drugs, as suggested by the fact that discontinuation of TNF-blocking agents tended to be more frequently due to an adverse event (and not inefficacy), and that none of them had been previously treated with rituximab.

We suggest that the CD28- T cell number reflects the bur-

den of prolonged previous immune stimulation, and that this is the reason that the efficacy of T cell costimulation-blocking by abatacept may be predicted by this measure. Indeed, many patients, although not achieving remission or good clinical response within 6 months, experienced some degree of response to abatacept and did not discontinue the treatment. A sizeable number of them continued to progressively improve, obtaining remission or good clinical response with longer duration of treatment (Figures 1 and 3). Interestingly, numbers of CD28- T cells at time of remission or response were not different in patients who achieved these positive results before or after 6 months, and they were in both cases significantly lower than those at baseline. This suggests that baseline numbers of CD28- T cells may predict the time needed to obtain the response up to prespecified criteria such as the DAS28-CRP, rather than being markers of responsiveness to abatacept. According to this hypothesis, when comparing patients

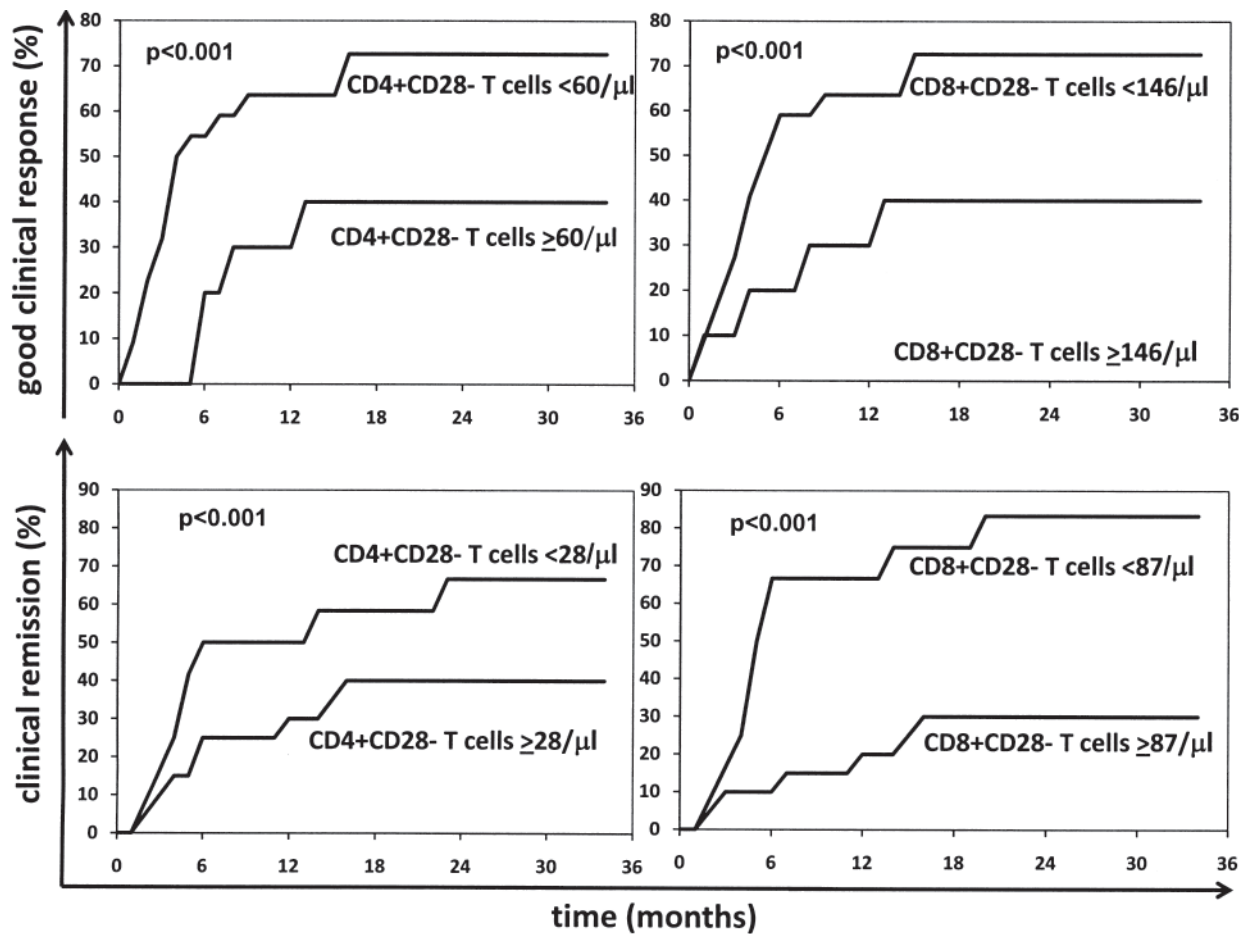


Figure 3. Kaplan-Meier cumulative probability of obtaining EULAR good clinical response or remission (DAS28-CRP < 2.6) in RA patients treated with abatacept with baseline CD4+ or CD8+ CD28- T cell numbers lower or higher than the cutoffs indicated by receiver-operator curves.

obtaining remission or good clinical response within 12 months, or at any time in therapy with patients not achieving these results, the predictivity of numbers of CD28- T cells was less evident.

We also suggest that the low number of circulating CD28- T cells observed at time of response to abatacept may reflect the efficacy of the costimulation block⁴. CD28- T cells generally display functional properties of differentiated effector and/or cytotoxic cells¹³, in particular producing large amounts of interferon- γ (IFN- γ)¹⁴. Little information is available on the *in vivo* biological effects of abatacept on T cells in patients with RA. However, data from both synovial tissue¹⁵ and serum studies¹⁶ point to reduction of IFN- γ expression and production as the most prominent effect of abatacept, with good correlation with drug efficacy. Since IFN- γ is produced mainly by effector CD8+ T cells, and the large majority of this population has a CD28- phenotype, these data suggest that the modulation of effector (CD28-) T cell function might be one of the mechanisms by which abatacept achieves its clinical effects.

There are limited data also on the effect of other biological therapies on the number of circulating CD28- T lymphocytes in RA. Studies in small numbers of patients have shown that

infliximab may decrease the number of circulating CD4+CD28- lymphocytes^{17,18}. However, there was no proof that such a decrease was correlated with clinical response. We are not aware of data on studies concerning CD8+CD28- cells or the influence of other biological agents on these cell populations. It is nevertheless possible that, in addition to abatacept, other therapies can induce changes of CD28- T cell populations. This may be particularly true for TNF-blocking agents, since *in vitro* downmodulation of CD28 on CD28+ T cells can be achieved by cultivating them in the presence of TNF- α ⁸. However, no study has yet evaluated the possible role of CD28- T cell numbers as a predictor of response to TNF-blocking agents.

The main limitation of our study was the small number of patients evaluated. However, we considered it worthy of report because of the novelty of the observation and its possible clinical relevance. In our opinion, further (possibly multi-center) studies on larger cohorts of patients are warranted to verify the predictive role of the test described here in the clinical response to abatacept treatment in patients with RA. Although this is not yet routinely done by hospital-based central laboratories, it is indeed a simple test for those with flow

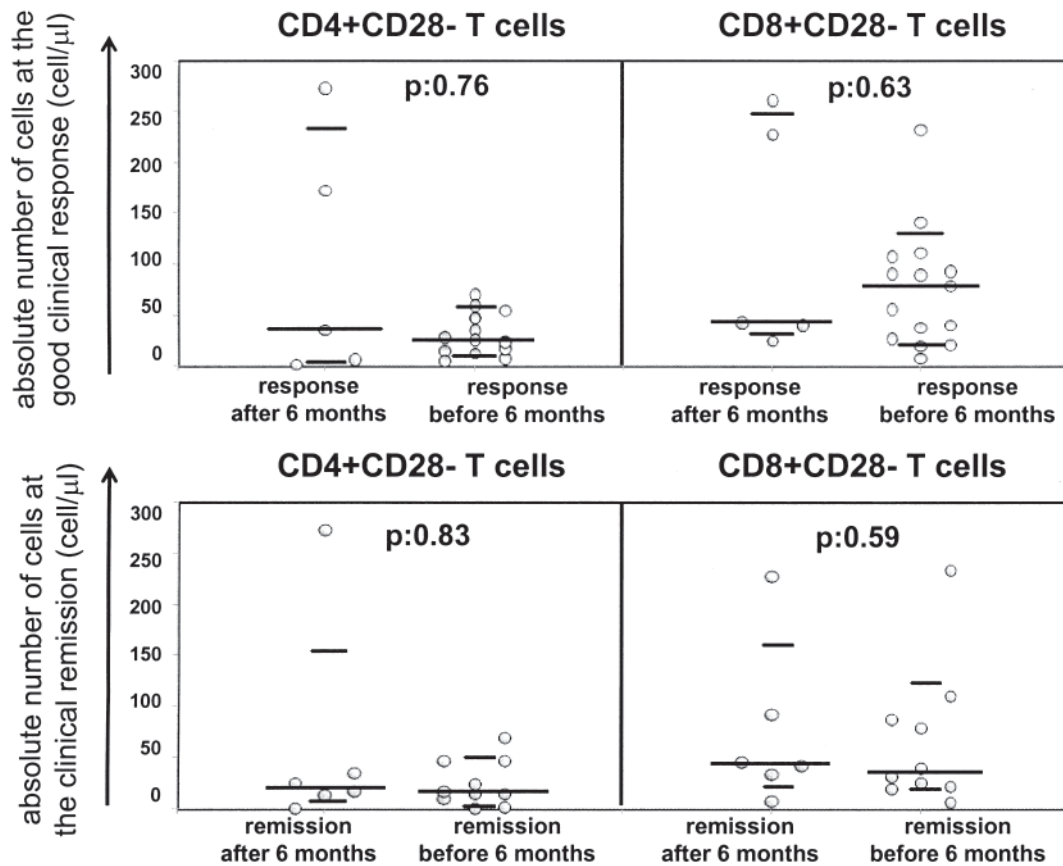


Figure 4. Comparisons of absolute numbers of CD4+ or CD8+ CD28- T cells at the time of EULAR good clinical response or remission (DAS28-CRP < 2.6) among RA patients treated with abatacept who achieved these results before or after 6 months.

cytometry facilities. If our results can be confirmed, this could become a routine test to assist clinicians in therapy decisions after the failure of the first TNF-blocking agent in cases of RA¹⁹.

REFERENCES

- Isaacs JD, Ferraccioli G. The need for personalised medicine for rheumatoid arthritis. *Ann Rheum Dis* 2011;70:4-7.
- Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med* 2003;349:1907-15.
- Schmidt D, Goronzy JJ, Weyand CM. CD4+ CD7- CD28- T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. *J Clin Invest* 1996;97:2027-37.
- Scarsi M, Ziglioli T, Airo' P. Decreased numbers of circulating CD28-negative T cells in patients with rheumatoid arthritis treated with abatacept are correlated with clinical response. *J Rheumatol* 2010;37:911-6.
- Martens PB, Goronzy JJ, Schaid D, Weyand CM. Expansion of unusual CD4+ T cells in severe rheumatoid arthritis. *Arthritis Rheum* 1997;40:1106-14.
- Gerli R, Schillaci G, Giordano A, Bocci EB, Bistoni O, Vaudo G, et al. CD4+CD28- T lymphocytes contribute to early atherosclerotic damage in rheumatoid arthritis patients. *Circulation* 2004;109:2744-8.
- Lake RA, O'Hehir RE, Verhoef A, Lamb JR. CD28 mRNA rapidly decays when activated T cells are functionally anergized with specific peptide. *Int Immunol* 1993;5:461-6.
- Bryl E, Vallejo AN, Matteson EL, Witkowski JM, Weyand CM, Goronzy JJ. Modulation of CD28 expression with anti-tumor necrosis factor- α therapy in rheumatoid arthritis. *Arthritis Rheum* 2005;52:2996-3003.
- Linsley PS, Bradshaw J, Urnes M, Grosmaire L, Ledbetter J. CD28 engagement by B7/BB-1 induces transient down-regulation of CD28 synthesis and prolonged unresponsiveness to CD28 signaling. *J Immunol* 1993;150:3161-9.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
- Wells G, Becker JC, Teng J, Dougados M, Schiff M, Smolen J, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis* 2009;68:954-60.
- Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36.
- Vingerhoets JH, Vanham GL, Kestens LL, Penne GG, Colebunders RL, Vandenbruaene MJ, et al. Increased cytolytic T lymphocyte activity and decreased B7 responsiveness are associated with CD28

- downregulation on CD8+ T cells from HIV-infected subjects. *Clin Exp Immunol* 1995;100:425-33.
14. Weyand CM, Goronzy JJ. T-cell responses in rheumatoid arthritis: systemic abnormalities-local disease. *Curr Opin Rheumatol* 1999;11:210-7.
 15. Buch MH, Boyle DL, Rosengren S, Saleem B, Reece RJ, Rhodes LA, et al. Mode of action of abatacept in rheumatoid arthritis patients having failed tumour necrosis factor blockade: a histological, gene expression and dynamic magnetic resonance imaging pilot study. *Ann Rheum Dis* 2009;68:1220-7.
 16. Marti L, Golmia R, Golmia AP, Paes AT, Guilhen DD, Moreira-Filho CA, et al. Alterations in cytokine profile and dendritic cells subsets in peripheral blood of rheumatoid arthritis patients before and after biologic therapy. *Ann NY Acad Sci* 2009;1173:334-42.
 17. Pawlik A, Ostanek L, Brzosko I, Brzosko M, Masiuk M, Machalinski B, et al. Therapy with infliximab decreases the CD4+CD28- T cell compartment in peripheral blood in patients with rheumatoid arthritis. *Rheumatol Int* 2004;24:351-4.
 18. Gerli R, Bartoloni-Bocci E, Shoenfeld Y. Effect of tumor necrosis factor a inhibition on CD28 surface expression on CD4+ T cells. *Arthritis Rheum* 2006;54:3060-1.
 19. Papaagoras C, Voulgari PV, Drosos AA. Strategies after the failure of the first anti-tumor necrosis factor alpha agent in rheumatoid arthritis. *Autoimmun Rev* 2010;9:574-82.