fresh synovial tissue biopsy samples from 3 RA patients as previously reported (8). Concentrations of MCP-1/CCL2 were determined using enzyme-linked immunosorbent assay (Quantikine human MCP-1 immunoassay; R&D Systems, Minneapolis, MN). Assays were performed according to the manufacturer's instructions. The effect of olmesartan on the induction of MCP-1/CCL2 was evaluated in the absence of serum. 293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (volume/volume) heat-inactivated fetal calf serum, penicillin, and streptomycin. Plasmids encoding 4κB-Luc have been described previously (9). 293T cells were transfected with 1 μg of reporter plasmid (p4κB-Luc) with lipofectamine (Gibco BRL, Gaithersburg, MD) as previously reported (9). Luciferase activity was measured with the Luciferase Assay System (Promega, Madison, WI). Transfection efficiency was monitored by *Renilla* luciferase activity using the pRL-TK plasmid (Promega) as an internal control. Western blot analysis was performed by standard methods. All incubations with antibodies were performed for 1 hour at room temperature. To study the effect of olmesartan on Ang II–induced degradation of IκBα, cells were treated with various concentrations of olmesartan for 10 minutes and stimulated with Ang II ($10^{-5}M$). An anti-IκBα antibody (SC-371; Santa Cruz Biotechnology, Santa Cruz, CA) was used for detection of IκBα.

Ang II–induced MCP-1/CCL2 production from RASFs was observed, and olmesartan suppressed this induction in a dose-dependent manner (Figure 1A). As previously reported with smooth muscle cells, Ang II induced NF-κB–dependent transcriptional activation, and inhibition of this activity by olmesartan was dose-dependent (Figure 1B). To confirm the stimulation of NF-κB in RASFs by Ang II we analyzed degradation of IκBα, which is an inhibitory binding protein of NF-κB. As shown in Figure 1C, Ang II induced degradation of IκBα, and olmesartan inhibited this degradation.

These results provide evidence that Ang II activates NF-κB in RASFs following the induction of MCP-1/CCL2, which is important in the development of arthritis. The ARB olmesartan was found to suppress this process. Therefore, a possible explanation for part of the antiarthritic activity of olmesartan observed by Sagawa et al may be related to action via an Ang II–AT_1–NF-κB–MCP-1/CCL2 signaling pathway.

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1. Ruiz-Ortega M, Lorenzo O, Ruperez M, Konig S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor κB through AT(1) and AT(2) in vascular smooth muscle cells: molecular mechanisms. Circ Res 2000;86:1266–72.
2. Walsh DA, Suzuki T, Knock GA, Blake DR, Polak JM, Wharton J. AT1 receptor characteristics of angiotensin analogue binding in human synovium. Br J Pharmacol 1994;112:435–42.
3. Sagawa K, Nagatani K, Komagata Y, Yamamoto K. Angiotensin receptor blockers suppress antigen-specific T cell responses and ameliorate collagen-induced arthritis in mice. Arthritis Rheum 2005;52:1920–8.
4. Koch AE, Kunkel SL, Harlow LA, Mazaraakis DD, Haines GK, Burdick MD, et al. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J Clin Invest 1992;90:772–9.
5. Akahoshi T, Wada C, Endo H, Hirota K, Hosaka S, Takagishi K, et al. Expression of monocyte chemotactic and activating factor in rheumatoid arthritis: regulation of its production in synovial cells by interleukin-1 and tumor necrosis factor. Arthritis Rheum 1993;36:762–71.
6. Huang DR, Wang J, Kivisakk P, Rollins BJ, Ransohoff RM. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. J Exp Med 2001;193:713–26.
7. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. J Exp Med 2000;192:1075–80.
8. Okamoto H, Iwamoto T, Kotake S, Momohara S, Yamanaka H, Kamatani N. Inhibition of NF-κB signaling by fenofibrate, a peroxisome proliferator activated receptor-α ligand, present a therapeutic strategy for rheumatoid arthritis. Clin Exp Rheumatol 2005;23:323–30.
9. Tetsuka T, Uranishi H, Sanda T, Asamitsu K, Yang JP, Wong-Staal F, et al. RNA helicase A interacts with nuclear factor κB p65 and functions as a transcriptional coactivator. Eur J Biochem 2004;271:3741–51.

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Overestimation of the prevalence of ankylosing spondylitis in the Berlin study: comment on the article by Braun et al

To the Editor:

Braun et al's carefully designed epidemiologic study from Berlin, Germany, on the prevalence of spondylarthropathies (SpA) and ankylosing spondylitis (AS) (1), was reported in your journal and has been cited extensively. We would like to point out some important flaws in the estimation of the prevalence rates in that report that have been previously overlooked.

In the above-mentioned study, SpA was diagnosed (using the European Spondylarthropathy Study Group criteria [2]) in 19 of 140 B27-positive subjects (13.6%) as opposed to only 1 of 133 B27-negative subjects (0.7%). On the basis of a B27 frequency of 9.3% in the general population of Berlin, and a total adult population (ages 18–65) of 2,367,507 individuals, the estimated prevalence of SpA was calculated as follows: B27-positive population of Berlin ages 18–65 = 220,178; B27-negative population of Berlin ages 18–65 = 2,147,329; SpA prevalence in B27-positive blood donors = 13.6%; SpA prevalence in B27-negative blood donors = 0.7%; prevalence of SpA = (220,178 × 13.6%) + (2,147,329 × 0.7%)/2,367,507 = 1.9%.

Despite a marked male predominance (67%) in the sample population, Braun et al seem to have made no adjustment for this when extrapolating their data to the general population. They also made no adjustment for any possible differences between the age distributions of the sample and target populations. Moreover, the prevalence of SpA in B27-negative subjects (0.7%) was obtained from a sample size of only 133 subjects. When the expected prevalence is so low, a sample size of 2,963 B27-negative subjects is required to make an accurate estimate at a 95% confidence level, with a worst acceptable frequency of 0.4% (EPIINFO 6.0 software). Thus, the projected prevalence rate of SpA calculated in their report may not reflect the true prevalence of SpA in the target population.

Nine of 20 SpA patients met the classification criteria for AS, using the modified New York criteria (3), and all 9 of these patients were B27 positive. Thus, 9 of 140 B27-positive subjects (6.4%) had AS. But the method for calculation of the general prevalence of AS is not clearly elucidated in their report. The authors may have simply calculated that, since AS patients comprised 45% of the 20 SpA patients, AS represents 45% of the prevalence rate of SpA (1.9% × 45% = 0.86%). Such an approach, however, presupposes that B27 associations with SpA and AS are similar. According to such a calculation, there should be 13,475 B27-positive AS patients (220,178 × 13.6% × 45%) and 6,764 B27-negative AS patients (2,147,329 × 0.7% × 45%) in Berlin. This will yield a B27 association with AS of 67% (13,475 of 20,239), and this association will be equal to that found for SpA patients who did not have AS. However, it is well known that the association of AS with HLA–B27 is much stronger than that with other forms of SpA (4).

A better and more correct approach would be to calculate the total number of B27-positive patients with AS (220,178 × 6.4% = 14,091) and then estimate the overall prevalence of AS, assuming that the B27-positive patients comprised 90% of all AS patients in Berlin (since ~90% of German AS patients are positive for B27). This approach would show the prevalence rate of AS to be 0.66% (15,657 of 2,367,507). But even with this approach, one should have first standardized the prevalence rate according to the structure of the target population.

For the above reasons, we believe that the prevalence rates reported in the Berlin study should be revised accordingly.

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1. Braun J, Bollow M, Remlinger G, Eggers U, Rudwaleit M, Distler A, et al. Prevalence of spondylarthropathies in HLA–B27 positive and negative blood donors. Arthritis Rheum 1998;41:58–67.
2. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991;34:1218–27.
3. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnositc criteria for ankylosing spondylitis: a proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
4. Khan MA. Update on spondyloarthropathies. Ann Intern Med 2002;136:896–907.

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Reply

To the Editor:

We would like to thank Drs. Akkoc and Khan for their careful review of our 1998 study on the prevalence of AS and the whole group of spondylarthropathies. Their recalculation of our data suggests an overestimation of the prevalence of AS in our previous report and they propose a more conservative value of 0.66%. There are several factors of possible relevance for such calculations: 1) HLA–B27 association of the subset; 2) HLA–B27 background in the population; 3) structure of the target population; 4) sex distribution; 5) sample size (number of HLA–B27–negative subjects); 6) ascertainment of diagnosis.

At the time we performed the study, we did not adjust for these factors because we thought that, with this type of study that includes blood donors, only rough estimates are possible and more detailed calculations may not be useful. In this regard, we agree that the calculation based on HLA–B27–negative donors is very risky. Nevertheless, since Drs. Akkoc and Khan have now analyzed our report in some detail, we have taken the challenge to recalculate the original numbers.

In our study, which was performed in Berlin, SpA was diagnosed in 20 of 273 individuals: 19 of 140 B27-positive individuals (13.6%) and 1 of 133 B27-negative individuals (0.7%) (15 male, 5 female). AS was diagnosed in 9 individuals (7 male, 2 female; 45%), undifferentiated SpA in 7 (5 male, 2 female; 35%), psoriatic arthritis in 3 (2 male, 1 female; 15%), and chronic reactive arthritis in 1 (male; 5%). The relative risk of developing AS in B27-positive individuals was calculated to be 6.43%.

Using the above results along with published data on the male:female ratio in the population (1) (Table 1), we calculated standardized prevalence rates with 95% confidence intervals (95% CIs) and standard errors (SE), as follows: AS = ([7/95] × 4.72) + ([2/45] × 4.58) + 0 + 0 = 0.55% (SE 0.19%, 95% CI 0.18–0.92%); SpA = ([14/95] × 4.72) + ([5/45] × 4.58) + ([1/88] × 46.05) + 0 = 1.73% (SE 0.6%, 95% CI 0.56–2.9%).

Thus, we agree with Drs. Akkoc and Khan that the prevalence rates calculated in 1998 represented an overestimation. However, as already discussed, there are limitations to the approach of looking at "healthy" blood donors who are artificially enriched with HLA–B27. Nevertheless, the strength of that approach was that most patients were clinically examined and many underwent magnetic resonance imaging, which is now considered a useful tool, not only for the early diagnosis of SpA, but also for assessment of disease activity (2,3).

Despite the discovery in 1973 of a strong genetic link of HLA–B27 to AS and to the group of spondylarthropathies overall (4) we still do not know the nature of the obvious influence of HLA–B27 on these rheumatic diseases. The HLA–B27 background prevalence in a population is known to have some impact on the prevalence of AS. The history of prevalence studies in AS started in the 1960s. In 1975, the first blood donor study was published (5), in which an overall prevalence of AS of 1.0–1.5% was calculated. This high number was clearly not reproduced in a Dutch population-based study (6), which had an HLA–B27 background of ~8%. Since definite AS was rarely found, it was calculated that 1.3% of the HLA–B27–positive population, in comparison with only 0.1% of the total population, had AS. In contrast, an epidemiologic survey in northern Norway (7) revealed a prevalence of definite AS of 1.1–1.4%, with an HLA–B27 background of ~15%. It was calculated that 6.7% of the B27-positive individuals had AS. This proportion is consistent with our findings (Table 1).

There are several other publications on the subject, but we have only mentioned some very recent studies for comparison. The age- and sex-adjusted prevalence of AS in Greece was found to be 0.3% (8). From France (9,10), overall prevalence rates of SpA of 0.47% and 0.30% were reported, which are quite similar to the rates for rheumatoid arthritis. In