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From undifferentiated arthritis to rheumatoid arthritis : epidemiology, immunology and early intervention

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**From undifferentiated arthritis to
rheumatoid arthritis:
epidemiology, immunology and
early intervention**

Henrike Gillet-van Dongen

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epidemiology, immunology and early intervention**

PROEFSCHRIFT

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Prof. dr. R.R.P. de Vries
Prof. dr. F. Koning
Dr. C.F. Allaart

'On ne voit bien qu'avec le coeur. L'essentiel est invisible pour les yeux.'

Antoine de Saint-Exupéry (1900 - 1944)

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CHAPTER 1

Introduction

1 Introduction

2 3 Arthritis

4 The clinical diagnosis of arthritis is characterised by warm, painful and swollen joints
5 in one or more areas. The most common chronic inflammatory joint syndrome is rheu-
6 matoid arthritis, which affects approximately 1% of the population (1). In rheumatoid
7 arthritis, the arthritis eventually leads to destruction of the joint. Distinguishing rheu-
8 matoid arthritis from other types of arthritis can be difficult. In the 1950s, a committee
9 of the American Rheumatism Association (ARA) categorised arthritis based on expert
10 opinions, epidemiologic surveys and clinical cases. Diagnostic criteria for possible, prob-
11 able and classical or definite rheumatoid arthritis were proposed (2). In 1987 the criteria
12 for rheumatoid arthritis were reassessed (3). These criteria are still used to define distinct
13 research populations. **Table 1** shows the criteria for probable rheumatoid arthritis from
14

15 **Table 1.** Criteria for rheumatoid arthritis and probable rheumatoid arthritis

16 No.	17 Criterion
18 1	19 Morning stiffness.
20 2	21 Pain on motion or tenderness in at least one joint (observed by a physician).
22 3	23 Swelling (soft tissue thickening or fluid, not bony overgrowth alone) in at least one joint (observed by 24 a physician).
25 4	26 Swelling (observed by a physician) of at least one other joint (any interval free of joint symptoms 27 between the two joint involvements may not be more than 3 months).
28 5	29 Symmetrical joint swelling (observed by a physician) with simultaneous involvement of the same 30 joint on both sides of the body (bilateral involvement of midphalangeal, metacarpophalangeal or 31 metatarsophalangeal joints is acceptable without absolute symmetry). Terminal phalangeal joint 32 involvement will not satisfy this criterion.
33 6	34 Subcutaneous nodules (observed by a physician) over bony prominences, on extensor surfaces or in 35 juxta-articular regions.
36 7	37 X-ray changes typical of rheumatoid arthritis (which must include at least bony decalcification 38 localized to or greatest around the involved joints and not just degenerative changes). Degenerative 39 changes do not exclude patients from any group classified as rheumatoid arthritis.
8	9 Positive agglutination test - demonstration of the "rheumatoid factor" by any method which, in 10 two laboratories, has been positive in not over 5% of normal controls - or positive streptococcal 11 agglutination test.
9	12 Poor mucin precipitate from synovial fluid (with shreds and cloudy solution).
10	13 Characteristic histologic changes in synovial membrane with three or more of the following: marked 14 villous hypertrophy; proliferation of superficial synovial cells often with palisading; marked infiltration 15 of chronic inflammatory cells (lymphocytes or plasma cells predominating) with tendency to form 16 "lymphoid nodules"; deposition of compact fibrin, either on surface or interstitially; foci of cell necrosis.
11	17 Characteristic histologic changes in nodules showing granulomatous foci with central zones of cell 18 necrosis, surrounded by proliferated fixed cells, and peripheral fibrosis and chronic inflammatory cell 19 infiltration, predominantly perivascular.

(A) Diagnostic criteria for probable rheumatoid arthritis (2). This diagnosis requires three of the criteria.
In at least one of the criteria number 1 through 5 the joint signs or symptoms must be continuous for at
least six weeks.

No.	Criterion	Definition
1	Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2	Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician. The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints
3	Arthritis of hand joints	At least 1 area swollen (as defined above) in a wrist, MCP, or PIP joint
4	Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body (bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry)
5	Rheumatoid nodules	Subcutaneous nodules, over bony prominences, on extensor surfaces, or in juxtaarticular regions, observed by a physician
6	Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7	Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

(B) 1987 Criteria for the classification of acute arthritis of rheumatoid arthritis (3). For classification purposes, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 of these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weeks. Patients with 2 clinical diagnoses are not excluded. Designation as classic, definite, or probable rheumatoid arthritis is not to be made.

1958 and for rheumatoid arthritis from 1987. If the arthritis does not fulfil any criteria belonging to a disease description by the American College of Rheumatology and other diagnoses involving arthritis are ruled out, you are left with an undifferentiated arthritis. This undifferentiated arthritis can be an early stage of rheumatoid arthritis which has not been recognised yet as such. To study amongst others the epidemiology of arthritis, early arthritis clinics were set up throughout the world. In Leiden, an Early Arthritis Clinic was started in 1993. From the first 1000 patients who were included 37% was classified as having an undifferentiated arthritis (4). After 1 year, the diagnosis of the patients who presented with an undifferentiated arthritis was reassessed (5). About 30% of these patients had no signs of arthritis after 1 year. However, 28% of the patients were recognised within 1 year to have rheumatoid arthritis.

Monitoring arthritis

Rheumatoid arthritis is characterised by progressive destruction of the joints, either slowly or rapidly. These destructions eventually lead to disability. To monitor efficacy of treatments different methods were developed to evaluate the intensity of a (destructive) rheumatoid arthritis. The main methods that are used in the next chapters are described in this paragraph.

1 The disease activity score (DAS) consists of both objective and subjective items. It was
 2 developed using a discriminant analysis based on the decision of rheumatologists to
 3 start or switch an anti-rheumatic drug in combination with parallel blind clinical assess-
 4 ments of research nurses (6;7). The DAS is calculated as follows:

$$\begin{aligned}
 5 & \\
 6 \text{ DAS} &= 0.53938 * \text{sqrt (Ritchie score)} \\
 7 &+ 0.06465 * (\text{number of swollen joints}) \\
 8 &+ 0.330 * \ln (\text{erythrocyte sedimentation rate}) \\
 9 &+ 0.00722 * (\text{patient global assessment})
 \end{aligned}$$

10
 11 In the Ritchie articular index 53 joints are scored for tenderness. Forty-four joints are scored
 12 for swelling (8). For most rheumatologists a DAS of more than 2,4 means an active disease
 13 state that is high enough to intensify medication (9). A DAS of 1,6 is used as a cut off for
 14 remission (10). To simplify the calculation of the DAS for daily practice, substitutes were de-
 15 veloped. The original DAS with the mentioned 4 variables was reduced to a DAS with three
 16 variables in which the patient global assessment value was replaced by 0,224 (7). A modified
 17 DAS that included 28-joint counts instead of 44 (8) and a simplified disease activity index
 18 were validated as well (11). In studies described in this thesis the original DAS was used.

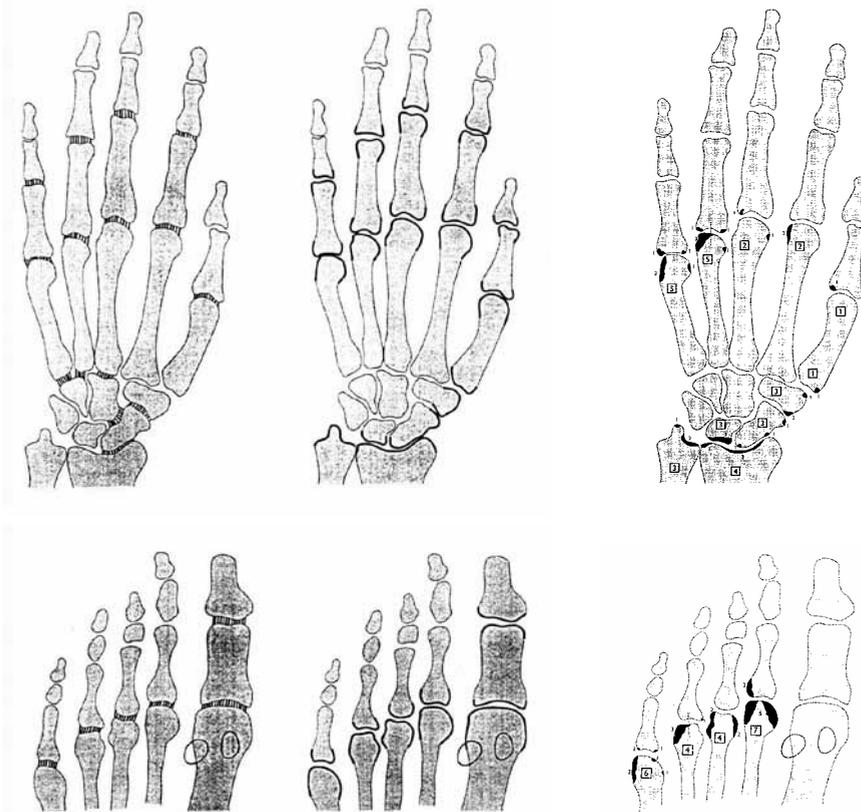
19 A more objective method for measuring the destructiveness of rheumatoid arthritis is
 20 scoring the radiographic joint damage. There are different scoring methods to evaluate
 21 radiographic joint damage. In the projects described in this thesis the Sharp-van der
 22 Heijde modification scoring method was used. This method focuses on the small joints
 23 in the hands, the wrists and the feet. Joint space narrowing is scored in 42 joints, and the
 24 distribution of erosions is scored in 44 joints, as is depicted in **Figure 1** (12).

25 The health assessment questionnaire (HAQ), arthritis impact measurement scales
 26 (AIMS), rheumatoid arthritis disease activity index (RADAI) and modifications on them
 27 are patient centered assessments focussing on the impact of the arthritic disease in daily
 28 life (13-16). For example, performance of physical activities is graded by the patient, and
 29 the general well being and disease activity are rated on visual analogue scales (VAS)
 30 from 1 to 10 by the patient or the physician.

31 32 **Evidence for early treatment**

33 There is no curative treatment for rheumatoid arthritis. Over the past twenty years,
 34 the treatment of rheumatoid arthritis has significantly changed with the introduction
 35 of disease-modifying anti-rheumatic drugs (DMARDs) and later on the introduction
 36 of biologicals. Before 1994 most patients who were diagnosed with RA started with a
 37 non-steroid anti-inflammatory drug (NSAID). NSAIDs give symptom reduction, but are
 38 not thought to interfere with the underlying disease process in contrast to DMARDs.
 39 Moreover, it was shown that patients with RA who started with a DMARD as soon as

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23 **Figure 1.** Scoring of hands and feet according to the Sharp-van der Heijde scoring method
24 Joints and sites scored for joint space narrowing (left panel), joints and surfaces of the joints scored for
25 erosions (middle panel), examples of scoring erosions according to the joint surface involved
26 with the van der Heijde modification for hands and feet. The small numbers indicate how an erosion is
27 scored and the numbers in the boxes give the total score for that joint. Reproduced from *Van der Heijde*
28 *DMFM. Plain X-rays in rheumatoid arthritis: overview of scoring methods, their reliability and applicability.*
29 *Bailliere's Clinical Rheumatology* 1996 (12).

30 the diagnosis was made had less disease activity and less radiographic joint damage
31 or progression of it compared to patients who first started with NSAIDs, and the best
32 results were yielded in the first year of treatment (17-19). After 10 years, the beneficial
33 effects of immediate treatment with DMARDs was even reflected in a lower need for
34 joint surgery (20). Taking this knowledge into account, it was proposed that recognizing
35 rheumatoid arthritis in an early phase is crucial to the disease outcome on the long
36 term, a so-called window of opportunity (**Figure 2**). Treatment strategies have only been
37 developed for patients in the clinical phase who are recognised to have rheumatoid
38 arthritis. The window of opportunity actually lies in recognising individuals who are
39 at risk for developing rheumatoid arthritis in the preclinical phase and in recognising

1 patients with an undifferentiated arthritis who actually have rheumatoid arthritis, but
2 have no joint destruction yet.

3 4 **Pathophysiology**

5 Rheumatoid arthritis is classified as an autoimmune disease, in which leucocytes attack
6 the joints, leading to inflammation and resulting in destruction of the joint. Different fac-
7 tors are thought to be involved in the pathophysiology of rheumatoid arthritis. Genetic
8 susceptibility and environmental factors can create the conditions in which harmful T
9 cell responses are activated that are also able to provide help to B cells with subsequent
10 production of antibodies. Past decades different parts of this cascade have been under
11 investigation.

12 13 **Genetic background**

14 The influence of a genetic component for rheumatoid arthritis is emphasised in a three-
15 to fourfold higher concordance percentage of disease in monozygotic twins compared
16 to dizygotic twins and the total genetic contribution to rheumatoid arthritis is estimated
17 to be 50-60% (21-24). A wide variety of candidate genes has been investigated for their
18 influence on susceptibility to and severity of rheumatoid arthritis. The most prominent
19 genetic risk factor found so far are the human leucocyte antigen (HLA) class II molecules.
20 An approach to understanding the molecular genetics of susceptibility to rheumatoid
21 arthritis is the shared epitope hypothesis (25). The initiation of an immune response re-
22 quires T cell activation, and such activation requires the presence of antigen presented
23 by HLA class II molecules on antigen presenting cells. At the level of protein structure,
24 certain HLA-DRB1 alleles share an amino acid sequence in the beta-sheet of the peptide-
25 binding groove of the HLA molecule, the shared epitope. It is thought that the shared
26 epitope containing HLA-molecules are important for the presentation of arthritogenic
27 antigens. The shared epitopes, QRRAA (DR1), QKRAA (DR4) and RRRRAA (DR10), are not
28 only associated with susceptibility to rheumatoid arthritis, but also with a more pro-
29 gressive disease course (26;27). The contribution of the presence of the shared epitope
30 counts for 30% of the genetic aspects of rheumatoid arthritis (28). However, the puta-
31 tive peptides that fit in the groove and are directly involved in the cause of rheumatoid
32 arthritis have not been discovered so far.

33 34 **T cells**

35 The involvement of HLA class II molecules and the presence of joint antigen-directed
36 T cells suggest a role for CD4+ T cells (29). After arising from bone marrow stem cell
37 precursors, progenitor cells migrate to the thymus, where they will be matured into T
38 cells. In the thymus the T cells learn to distinguish self from non-self in the context of HLA
39

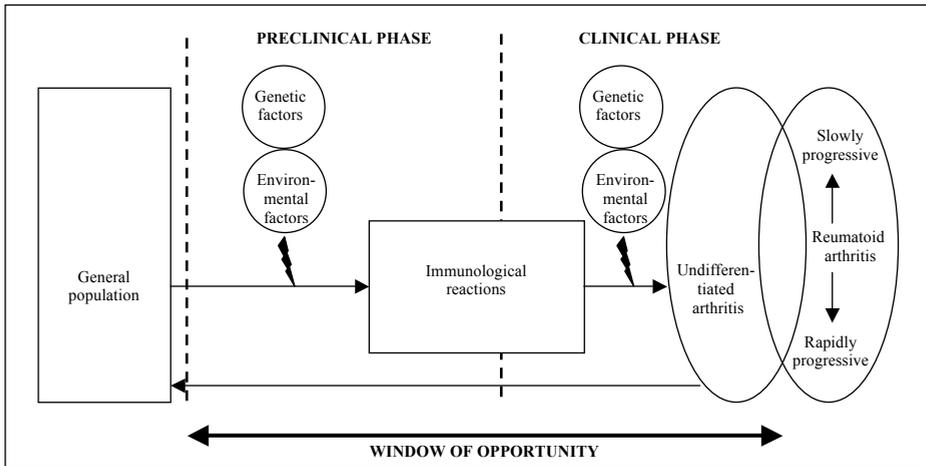


Figure 2. Working model for the disease course of arthritis

molecules. Firstly, those T cells that recognise self-HLA molecules are positively selected. Secondly, the negative selection process is said to eliminate the auto-reactive T cells.

Roughly, there are two types of T cells. Cytotoxic T cells, characterised by CD8 expression, recognise antigens in the context of HLA class I molecules, which are present on almost all nucleated cells. HLA class I molecules are loaded with intracellular proteins. If the CD8+ T cell is activated, it kills the antigen presenting cell by lysis. During these actions, pro-inflammatory cytokines like interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha and interleukin-2 (IL-2) are released.

T helper cells express CD4 and recognise antigens in the context of HLA class II molecules. Only antigen presenting cells like dendritic cells, monocytes, macrophages and B cells can process extracellular proteins to present them in HLA class II to CD4+ T cells. If the T cell is activated, it in turn activates a B cell that then starts to produce isotype-switched antibodies. This process is characterised by the release of IL-4 and IL-10.

However, this does not explain why regulatory or suppressor T cells exist and why or how autoimmune diseases develop. Different hypotheses about the origin of autoimmunity have been described varying from genetic defects involving the selection processes in the thymus to danger models in which damaged tissue releases danger signals that work as a costimulation factor for activating an auto-reactive process (30-33). The functional existence of regulatory T cells was first demonstrated in nude mice in which infusion with CD25+CD4+ T cells after transfer of CD25-CD4+ T cells prevented development of autoimmune diseases (34). CD25 is the alpha chain of the IL-2 receptor. It is highly expressed on activated T cells in the early phase of activation, often described as the CD25 bright cells in flowcytometric analysis, whereas regulatory T cells show an intermediate expression (35;36). Other markers like cytotoxic T lymphocyte antigen-4

1 (CTLA-4), glucocorticoid induced TNF receptor (GITR) and foxP3 have been used to
2 describe regulatory T cells, but are also not specific. The regulatory T cell response is
3 characterised by the release of anti-inflammatory cytokines like IL-10 and tumour
4 growth factor (TGF)-beta (37;38). Thus, T cell-mediated immunoregulation does likely
5 play a role in immunologic self-tolerance but exact characterisation of the regulatory T
6 cells remains difficult, especially in a human setting.

7 8 **Autoantibodies**

9 The presence of autoantibodies is also a reason to consider rheumatoid arthritis an auto-
10 immune disease. The positive predictive value of the presence of certain antibodies aims
11 at another phase of the immune response that may play a role in the pathophysiology of
12 rheumatoid arthritis. Rheumatoid factor (RF) is an antibody that is directed to the Fc part
13 of immunoglobulins. The presence of IgM-RF is part of the 1987 criteria for rheumatoid
14 arthritis. However, RF is also detected in other autoimmune diseases and healthy indi-
15 viduals. For diagnosing rheumatoid arthritis, the sensitivity of RF ranges from 60 to 70%;
16 the specificity from 80 to 90% (39).

17 Anti-citrullinated protein antibodies (ACPAs) are much more predictive for rheuma-
18 toid arthritis. In the Leiden EAC, 93% of the patients with ACPAs who presented with
19 undifferentiated arthritis were diagnosed with rheumatoid arthritis within 3 years (40).
20 Furthermore, patients with ACPA-positive rheumatoid arthritis had more erosive disease
21 than patients who had no ACPAs. Citrullination facilitates the degradation process
22 in a cell and is a normal physiologic process in the presence of inflammation (41). A
23 calcium-ion influx leads to activation of the peptidylarginine deiminase (PAD) enzyme,
24 which converts arginine into citrulline. The formation of antibodies against citrullinated
25 proteins is not physiological and is mainly found in rheumatoid arthritis (42). ACPAs
26 can be present up to 14 years upon diagnosing rheumatoid arthritis (43;44). During the
27 last years it has become clear that the genetic and environmental risk factors for ACPA-
28 positive and ACPA-negative disease differ. Moreover, the histology differs and, as stated
29 above, the clinical outcome differs. This has led to the awareness that subclassification of
30 rheumatoid arthritis in ACPA-positive and ACPA-negative disease is appropriate (45-47).

31 32 **Outline of this thesis**

33 The research projects described in this thesis are divided into two themes: part I involves
34 mainly epidemiological and clinical aspects of patients with undifferentiated arthritis
35 and part II involves immunological aspects of patients with rheumatoid arthritis.

36 The aim in **part I** of this thesis is to describe the epidemiology of undifferentiated ar-
37 thritis, to gather more evidence for early intervention, and to predict disease outcome. In
38 the Leiden EAC 37% of the patients who presented with arthritis were classified as having
39 an undifferentiated arthritis (4). In **chapter 2**, the incidence of undifferentiated arthritis

1 in several early arthritis registries in other parts of the world is described. Some patients
2 with rheumatoid arthritis present with a full blown rheumatoid arthritis while others
3 present themselves with clinically an undifferentiated arthritis. To characterise this dif-
4 ferent onset of disease, the long-term outcome of patients with such a 'slow onset' were
5 compared to the long-term outcome of patients who present with rheumatoid arthritis.
6 The results are described in **chapter 3**. In rheumatoid arthritis evidence for justification
7 of early aggressive treatment is accumulating. Starting treatment for rheumatoid arthri-
8 tis in patients with undifferentiated arthritis, the window of opportunity, has not been
9 performed before. The PROMPT study was the first randomised controlled trial in which
10 patients with undifferentiated arthritis were treated with a DMARD, methotrexate. The
11 results of the PROMPT-study are presented in **chapter 4**. In the PROMPT-study, the DAS-
12 score was used to evaluate the disease activity in patients with undifferentiated arthritis.
13 Based on the DAS-score the intensity of the study medication was maintained or raised.
14 However, this score system was designed for patients with rheumatoid arthritis. In **chap-**
15 **ter 5**, the DAS in patients with undifferentiated arthritis is discussed, investigated and
16 validated. Not all patients who were included in the PROMPT study had a very early stage
17 of rheumatoid arthritis. Before a patient is exposed to a treatment with methotrexate or
18 another DMARD or even more, a combination of DMARDs, the indication for such a treat-
19 ment should be carefully evaluated. Actually, you would like to predict which patient with
20 undifferentiated arthritis will benefit from an early treatment and who will go spontane-
21 ously in remission or will never develop the clinical syndrome of rheumatoid arthritis.
22 Therefore, a rule to predict disease outcome in patients with recent-onset undifferenti-
23 ated arthritis was developed and validated in the PROMPT study. This rule is presented in
24 **chapter 6**. ACPAs have a strong predictive value in predicting the probability of having
25 a very early stage of rheumatoid arthritis in patients presenting with undifferentiated
26 arthritis. Having identified an individual with an indication for treatment with second-
27 line anti-rheumatic drugs, you are left with a palette of DMARDs and combinations of
28 DMARDs. Tailor-made treatments for each stage of disease focused on individual needs
29 would be the best. In **chapter 7** the response to methotrexate treatment is related to the
30 pre-treatment serum levels of ACPA in patients with undifferentiated arthritis.

31 In **part II**, the accent lies on the immunological background of rheumatoid arthritis and
32 possibilities for intervention. Biologicals, like anti-TNF-alpha, have had a major impact on
33 the outcome of rheumatoid arthritis. However, it is unclear how TNF-alpha influences the
34 immune response in patients with rheumatoid arthritis. In **chapter 8** the effect of anti-
35 TNF-alpha on regulating the immune response in patients with rheumatoid arthritis is
36 described. In understanding and identifying the underlying immunological changes in
37 healthy individuals to processes resulting in rheumatoid arthritis, T-cell responses against
38 a human cartilage protein, HC-gp39, and in particular the presence of regulatory T cells
39 are investigated in **chapter 9**. As HC-gp39 seemed to be a usefull target in an attempt to

1 change the pro-inflammatory response in patients with rheumatoid arthritis, a phase I co-
2 hort study was performed by others (48). T cells derived from patients included in this study
3 were analysed for their immunological response. However, the results were not conclusive.
4 In **chapter 10** the results of this analysis and of this thesis are summarized and discussed.

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CHAPTER 2

Undifferentiated arthritis - Disease course assessed in several inception cohorts

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1 Abstract

2
3 The prognosis of patients with undifferentiated arthritis (UA) may vary from self-limited
4 to severe destructive rheumatoid arthritis (RA). Because early aggressive treatment
5 might offer an effective means to slow disease progression in RA, it is important to
6 identify UA patients who will develop RA and treat them as early as possible. At the same
7 time, inappropriate treatment of patients with a more benign disease course should be
8 avoided. Here, an overview is given of the characteristics and numbers of patients with
9 UA who evolve into RA.

10 UA is defined as any arthritis that has the potential for a persistent course, without ful-
11 filling the classification criteria for specific rheumatic disorders. To compare endpoints in
12 the different databases, the 1987 ACR criteria for RA were used.

13 In the nine databases employing a similar definition for undifferentiated arthritis,
14 the proportion of patients with UA that evolved into RA within 1 year varied from 6%
15 to 55%. These differences arise in large part from differences in the inclusion criteria
16 and in the definitions used for UA and RA. The data from the various cohorts support a
17 hypothesis that a considerable proportion of UA patients are actually patients with RA in
18 a very early stage. Controlled intervention studies with early antirheumatic treatment in
19 these patients are mandatory in order to provide further insight into the natural course
20 of UA and to define a treatment strategy that will successfully slow or prevent disease
21 progression.

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1 Introduction

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3 Several studies have indicated a beneficial effect of the early treatment of rheumatoid
4 arthritis (RA) to achieve a less severe disease course or even to induce remission (1-3).
5 The possible extra therapeutic benefit attainable in this early period in the disease has
6 been called the “window of opportunity”. Since the presentation pattern of RA varies
7 widely, it has been suggested that the treatment should be started as early as possible,
8 even before patients fulfil the American College of Rheumatology (ACR) criteria for RA
9 (4). Ideally, knowledge of prognostic factors in patients with undifferentiated arthritis
10 (UA) will allow the identification of those patients who will develop RA, so that the inap-
11 propriate treatment of patients who will not develop RA can be avoided. For this it is also
12 necessary to know the natural course of UA. The present review will attempt to describe
13 the natural course of UA as reported in early arthritis cohorts.

14 The first problem encountered in the search for the percentage of patients present-
15 ing with UA who will develop RA is the fact that UA is a non-validated description of a
16 phenotype. In clinical practice, all cases of arthritis that cannot be classified in one of the
17 accepted categories are referred to as *e causa ignota* or “undifferentiated”. For inclusion
18 in early arthritis cohorts, various definitions and criteria have been used for the early
19 phase of arthritis, which makes it difficult to compare the composition of the different
20 study groups. ‘Early arthritis’, ‘early RA’, and ‘undifferentiated arthritis’ are terms that are
21 currently in use to describe either arthritis that might evolve into RA or that has been
22 diagnosed early after onset of arthritis or even early in the disease course of definite
23 RA. Therefore, patients with UA are in general seen as those patients with the potential
24 for development of persistent inflammatory arthritis, including RA, but in whom a
25 recognized clinical pattern does not (yet) exist. In 1958 the American Rheumatism As-
26 sociation (ARA) identified criteria for ‘probable rheumatoid arthritis’ (5) as a distinction
27 from classical RA, but these criteria only define a subgroup of patients generally referred
28 to as having UA.

29 In this review, defining RA according to the classification criteria also has disadvan-
30 tages from a scientific viewpoint. The ACR criteria for RA were developed to identify
31 patients with established RA, and not for diagnostic purposes. In clinical practice, it is of
32 great relevance to distinguish patients on prognostic items such as persistent arthritis
33 or destructive arthritis. On the other hand, all intervention studies to date have been
34 based on fulfilment of the ACR criteria, and evidence that adequate treatment changes
35 the course of disease as well as the prognosis is available only in patients who meet the
36 ACR criteria. Therefore, notwithstanding the imperfect definitions of the phenotype for
37 clinical practice, it is important to assess what proportion of UA cases progress to RA, as
38 defined by the ACR criteria.

1 Inception cohorts

2
3 Early RA databases and their inclusion criteria are listed in **Table 1**. The databases marked
4 by an asterisk have included and described patients with UA. Only the latter databases
5 will be discussed. The other databases include 'early RA' patients who fulfilled the 1987
6 ACR criteria for established RA.

7 In Finland an early arthritis cohort was started in 1975 (6). Adults with one or more swollen
8 joints and a symptom duration of less than 6 months were referred to the hospital in
9 Heinola. Fortythree percent of the patients from this cohort had non-specific arthritis, de-
10 fined as probable RA according to the 1958 ARA criteria or arthritis not falling within any
11

12 **Table 1.** Early RA databases

13 Study group	14 Inclusion criteria	15 Study strategy and characteristics	16 N	17 Reference
18 Heinola Cohort/ 19 Rheumatism 20 Foudation 21 Hospital Cohort 22 (Finland) *	≥ 1 swollen joints disease duration ≤ 6 months age ≥ 16 years	prospective cohort referred by physicians of several health centres and hospitals follow-up after 1, 3, 8, 15, 20 and 25 years	442	(6)
23 Norfolk Arthritis 24 Register (UK) *	early inflammatory polyarthritis age ≥ 16 years ≥ 2 swollen joints symptom duration ≥ 4 weeks onset after January 1989	referred from GP and local rheumatologists yearly follow-up for at least 5 yrs patient visited at home		(10;22)
25 Leeds (UK) *	undifferentiated arthritis of the hands symptom duration < 12 months	patients from the Leeds Early Arthritis Clinic (n=1877) pyramid treatment strategy	97	(12)
26 Duesseldorf 27 (Germany) *	rheumatic symptoms duration ≤ 1 year age > 15 years	2-year prospective cohort study referred by GPs, internist, orthopaedic physicians	320	(13)
28 Austrian Early 29 Arthritis Registry *	inflammatory arthritis with ≥ 2 clinical criteria and ≥ 1 laboratory criterion duration of symptoms < 12 weeks	referred by GPs and internists to participating rheumatologists multi-centre (country-wide) every 3 months questionnaires		(14;16)
30 Wichita Arthritis 31 Centre (USA) *	undifferentiated polyarthritis syndrome or RA (ACR'87 criteria) disease duration ≤ 2 years	half of patients self-referred follow-up at least 13 months	506 (RA) 638 (UA)	(17)
32 ESPOIR Cohort 33 Study (France) *	certain or probable clinical diagnosis of RA UA that may develop into RA duration of symptoms < 6 months age 18-70 years ≥ 2 inflammatory joints for the past 6 weeks no DMARD use prior to inclusion	800 patients from the community 10 yrs follow-up		(18)
34 Amsterdam 35 (The Netherlands) *	≥ 2 swollen joints disease duration < 3 years	Patients from an early arthritis clinic	203	(19)

Study group	Inclusion criteria	Study strategy and characteristics	N	Reference
Leiden Early Arthritis Clinic (The Netherlands) *	any arthritis confirmed by rheumatologist symptom duration < 2 years no DMARD use prior to inclusion	referred by GPs follow-up at 2 weeks, 3 months and yearly		(20)
EURIDISS-Oslo (Norway)	RA (ACR'87 criteria) age 20-70 years disease duration ≤ 4 yrs	Norwegian part of international collaborative research effort follow-up at 1, 2 and 5 years	238	(23)
French Early Arthritis Cohort	RA (ACR'87 criteria) RA diagnosis < 1 year no DMARD use prior to inclusion	multi-centre referred from primary care follow-up 10 year		(18)
GIARA Registry Study Group (Italy)	RA (ACR'87 criteria)	aggressive RA registry	706	(24)
Jyväskylä Cohort (1983-1985) (Finland)	newly diagnosed RA (ARA'58 criteria)	follow-up 18-24 months	58	(6;25)
Jyväskylä Cohort (1988-1989) (Finland)	definite RA (ARA'58 criteria) and ≥ 2 criteria (ESR>20mm/hour, ≥ 6 joints with active RA, duration morning stiffness > 45 minutes) age 18-80 years symptom duration < 1 year	randomised, double blind, placebo controlled study on treatment with sulfasalazine follow-up at 4, 8, 12, 24 and 48 weeks	80	(6;26)
Central Finland RA database	(newly) diagnosed RA according to physician	all new patients with RA are referred to Jyväskylä Central Hospital	>2000	(6)
Helsinki Cohort (Finland)	RA (ACR'87 or revised ACR'87 criteria) symptom duration < 2 year no DMARD use prior to inclusion	prospective study on early aggressive therapy referred from primary care or private outpatients clinics	150	(6;27)
FIN-RACO study (Finland)	RA (ACR'87 criteria) symptom duration < 2 year age 18-65 year, ≥ 3 swollen joints and three of: ESR>28, CRP>19, morning stiffness.>29min, >5 swollen joints, >10 tender joints	multi-centre randomised trial on treatment strategies	199	(28)
CLEAR Registry (USA)	early RA disease duration < 2 years African-American		500	(29)
German early RA inception cohort	RA (ACR'87 criteria) age 21-75 years disease duration < 1 year	prospective, multi-centre study referred by GP, rheumatologist, arthritis care units follow-up at least 3 years		(30;31)

specific diagnostic group (7). The percentage of UA patients who developed RA was not mentioned. After 3 years 58% of the UA patients had no symptoms. Twenty-eight percent of the patients in this cohort met the 1987 ACR classification criteria for RA at inclusion.

From the same cohort, 32 patients were described with the diagnosis of non-classified monoarthritis, defined as swelling of a peripheral joint not due to trauma, degenerative

1 joint diseases or any other specific joint disease (8). Of those 32 patients, 2 (6%) had
2 rheumatoid factor (RF)-positive definite RA after a 3-9 year follow-up. In 29 patients the
3 diagnosis remained "non-classified" arthritis during follow-up.

4 In the Finnish cohort a group of 47 patients with recent onset RF-negative oligoarthritis
5 was also described (9). After 23 years of follow-up, reclassification of the diagnoses
6 revealed 1 patient with RA, 7 patients with erosions in the hands or feet, 1 patient with
7 systemic lupus erythematosus (SLE), 1 patient with ankylosing spondylitis, 2 patients
8 with "post-traumatic arthritis", 4 patients with osteoarthritis, and 6 patients with reactive
9 arthritis. The other 25 patients presumably still did not fulfil the criteria for a rheumatic
10 disease.

11 In the UK the Norfolk Arthritis Registry (NOAR) has been following patients with
12 early inflammatory polyarthritis who had been referred by general practitioners (GPs)
13 and local rheumatologists since January 1990, as described by Symmons *et al.* (10). All
14 adults with two or more swollen joints, lasting for at least 4 weeks, could be included.
15 The proportion of UA patients who developed RA was not mentioned in the published
16 data. However, Wiles *et al.* (11) described a study in which the ACR criteria were applied
17 cumulatively, meaning that once a criterion was fulfilled, this criterion was regarded as
18 positive in all subsequent assessments. In this study, 55% of the patients with a symptom
19 duration of less than 2 years satisfied the criteria for RA at inclusion as described above.
20 Sixty-seven percent fulfilled these criteria after one year.

21 Also from the UK, Quinn *et al.* (12) recently described a cohort of 97 patients with early
22 undifferentiated arthritis of the hands and a disease duration of less than 12 months
23 who were followed for 12 months. RA developed in 14% of the 97 UA patients. Thirty-six
24 percent had persistent synovitis (defined as the presence of 2 or more of the following:
25 joint swelling, joint tenderness or decreased range of motion) after 12 months, whereas
26 13% were in clinical remission. Only 54% of the patients could be diagnosed with a
27 specific rheumatic disease after a 12-month follow-up.

28 Initially these patients were included in a cohort of 1877 patients in the Leeds early
29 arthritis clinic of whom 56% had an inflammatory arthritis at inclusion; 50% of these
30 patients had RA and 23% had UA. Patients with UA were classified as having an inflam-
31 matory disorder where a specific rheumatic disease could not be diagnosed. It should be
32 noted that patients were eligible for inclusion in the study if they had a history sugges-
33 tive of inflammatory arthritis, but clinically detectable synovitis was not required. This
34 resulted in the observation that 47% of patients with UA had no synovitis at the time of
35 inclusion.

36 In Germany Huelsemann *et al.* (13) described a two-year prospective cohort study of
37 patients with "rheumatic symptoms" for less than 1 year's duration who were investi-
38 gated in an early arthritis clinic in Duesseldorf. The patients were sent to the tertiary
39 referral centre by general practitioners, internists and orthopaedic physicians. Of 320

1 patients who were investigated, 217 were classified as having inflammatory rheumatic
2 diseases. Of these 217 patients, 117 (54%) could not be diagnosed definitely and were
3 thus considered undifferentiated, and 39 (19%) were diagnosed as having RA. Sixty-
4 eight percent of the patients with UA presented with oligoarticular joint manifestations,
5 while 14% had a monoarticular and 18% had a polyarticular disease (5 or more joints).
6 Follow-up data 4 to 38 months after the initial symptoms were available for 28 patients
7 with UA. Fifteen (54%) of them had a complete remission, 8 patients had unchanged or
8 progressive unclassified disease and 2 (7%) were diagnosed with RA according to the
9 ACR 1987 criteria.

10 The Austrian early arthritis registry (Austrian Early Arthritis Action, EAA) (14) follows
11 patients with inflammatory arthritis whose symptoms began less than 12 weeks before
12 presentation and who fulfil at least 2 clinical criteria (absence of trauma, joint swelling in
13 at least 1 joint, joint pain in at least 1 joint, morning stiffness > 60 minutes) and at least
14 1 laboratory criterion (positive RF, ESR > 20 mm/hour, CRP > 5 mg/L, leucocytes > upper
15 limit of normal). Approximately 15% of the patients after 1 year still had no established
16 diagnosis and were classified as having UA. Sixty-five percent of the patients had RA
17 after 1 year, using the ACR 1987 criteria cumulatively as described in the NOAR (15).

18 In another paper, Machold *et al.* (16) describe 108 patients who had been followed
19 for at least 1 year. At inclusion, 31 patients (29%) had undifferentiated arthritis and 50
20 patients (46%) were diagnosed with RA. After 1 year, 17 of the UA patients (55%) were
21 diagnosed with RA. The diagnosis of RA was made if patients fulfilled the ACR 1987 crite-
22 ria, or if clinical examination revealed a polyarthritis of at least 6 weeks duration without
23 evidence of other inflammatory rheumatic diseases. In cases in which the diagnosis
24 could not be ascertained by the rheumatologist, the disease was classified as UA.

25 Wolfe *et al.* (17) followed 532 patients with undifferentiated arthritis at the Wichita
26 Arthritis Center who at presentation had a symptom duration of at least 2 years. Synovi-
27 tis was not required if the patient had other clinically suspected characteristics of RA in
28 the history, at physical examination or in laboratory results. 100% were followed up for
29 ≥ 13 months, 93% for ≥ 2 years and 87% for ≥ 3 years. 22% of the patients had no joint
30 swelling, and 6% had questionable swelling at the time of inclusion. Fifty-four percent
31 of the cases resolved, while 17% evolved into RA.

32 A French multi-centre cohort study (18) that includes patients with early arthritis with
33 a maximum duration of 6 months has recently been started. No data on this ESPOIR
34 cohort have been published yet. The study includes RA patients, probable RA patients
35 and patients with a clinical diagnosis of UA that may potentially develop into RA and
36 with at least two inflammatory joints for the past 6 weeks. UA patients with "no potential
37 to develop into RA" are excluded.

38 In a Dutch study by Jansen *et al.* (19), a group of patients from the Amsterdam early
39 arthritis clinic with peripheral arthritis involving at least 2 joints and a disease duration

1 of less than 3 years was followed in order to identify variables that could predict an
2 outcome of progressive disease after 1 year. In this study 27% (n=77) of the patients
3 were clinically diagnosed as having UA at inclusion and 72% (n=203) as RA. 42% of the
4 UA patients had oligoarthritis and 58% had polyarthritis. After one year 42% of the
5 patients with UA were categorized as progressive and 58% as mild, using radiographic
6 parameters and the HAQ score as criteria. Thirtyone percent of the progressive UA group
7 (n=10) fulfilled the ACR criteria for RA after one year. From the total UA group, 17% were
8 classified as having RA at 1 year.

9 The other Dutch cohort is the Leiden Early Arthritis Clinic, which includes patients
10 with any form of arthritis confirmed by a rheumatologist except gout, and a symptom
11 duration of 2 years or less (20). Out of 936 patients at inclusion, 346 (37%) were catego-
12 rized as having UA and 22% were diagnosed with RA. After one year of follow-up 32% of
13 the UA patients fulfilled the ACR 1987 criteria for RA. The percentage had increased to
14 40% at 3 years of follow-up (21).

16 Discussion

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18
19 We have reviewed inception cohorts with monoarthritis and polyarthritis to evaluate
20 what proportion of patients with UA progress to RA. In the various cohorts these pro-
21 portions varied considerably. This may be explained by the differences in referral and
22 recruitment procedures, inclusion criteria and, most notably, disease criteria between
23 the various cohorts. The reported proportion of patients with UA who progressed to
24 RA one year after inclusion range between 6% and 55%. However, in the cohorts that
25 required arthritis to be present at inclusion and that defined RA according to the ACR
26 1987 criteria, the proportions range from 17% to 32%.

27 The part of the Finnish early RA cohort in which only 6% of the patients with UA pro-
28 gressed to RA after a follow-up period of 3 to 9 years (8) probably represents a subgroup
29 of UA, defined as non-classified monoarthritis and RF negative oligoarthritis, and con-
30 sequently, a small group of patients is concerned (n = 32). Huelsemann *et al.* reported
31 that 7% of his patients with UA developed RA (13). However, at inclusion patients were
32 diagnosed based on clinical expertise and were not classified according to ACR criteria.
33 As only 18% of the UA patients at inclusion had a polyarticular disease, it is possible
34 that a certain proportion of the patients with polyarthritis at inclusion were prematurely
35 diagnosed as having RA. Therefore the proportion of UA patients who progressed to RA
36 might have been underestimated. Also, only 24% of the 117 patients with UA at inclu-
37 sion were followed. This suggests that these patients represent a subgroup of UA that
38 more often than not has a mild or self-limiting disease course.

1 Wolfe *et al.* reported that 17% of their UA patients progressed to RA after 3 years (17).
2 The inclusion of patients without synovitis in this cohort could have led to an under-
3 estimation of this value however. The same is true for the cohort described by Quinn
4 *et al.* (12). Jansen *et al.* (19) described a cohort of oligo- or polyarthritis patients, and
5 found a 17% progression from UA to RA. In a mixed population of monoand polyarthritis
6 patients, Van Gaalen *et al.* (21) reported that 32% progressed from UA to RA (diagnosis
7 according to the ACR 1987 criteria) within one year. An even higher rate of 55% was
8 described by Machold *et al.* (16). However, in that study not only patients who fulfilled
9 the ACR criteria were diagnosed as having RA, but also patients with polyarthritis for
10 more than 6 weeks without evidence of other inflammatory rheumatic diseases upon in-
11 vestigation. Therefore, the value of 55% could be an overestimation of RA in comparison
12 with other studies that focused only on the ACR criteria for diagnosing RA.

13 The findings of these cohort studies support the hypothesis that many patients with
14 UA are actually in the first stages of RA. Unpublished observations in the Leiden EAC
15 cohort indicate that patients whose UA evolved into RA within one year have, on aver-
16 age, the same prognosis as patients who presented with RA at baseline, as measured by
17 the rate of joint destruction, disease activity and functional status. Early treatment may
18 moderate the disease progression, possibly to the point that fewer patients develop RA
19 as defined by the ACR 1987 criteria. Ideally, patients with UA who will progress to RA
20 should be identified at presentation in order to receive early aggressive treatment.

21 Decisions to treat UA patients will depend on the likelihood that a patient will develop
22 RA. When this is high, it is worthwhile to start disease modifying anti-rheumatic drug
23 (DMARD) therapy immediately. Our review shows there is a 17-32% pre-test probability
24 that a patient with UA actually has RA. The question is what tests are available to obtain
25 a substantially higher post-test probability.

26 A great deal of research has already been carried out to try to identify predictors that
27 could be used for such a test. At present the most promising diagnostic tool appears to
28 be a test for anti-cyclic citrullinated peptide (CCP) autoantibodies. Van Gaalen *et al.* (21)
29 reported that in the Leiden EAC 93% of the patients with UA who were anti- CCP posi-
30 tive fulfilled the ACR 1987 criteria for RA within 3 years. The negative predictive value
31 was 75%. Furthermore, anti-CCP antibody testing was of little value in UA patients who
32 fulfilled none of the ACR 1987 criteria for RA, but had a significant additional value in
33 predicting the progression to RA in UA patients fulfilling one or more of these criteria
34 at presentation. As anti-CCP antibodies can be detected several years before the onset
35 of disease, Holers and Majka (32) proposed a model in which the development of anti-
36 CCP antibodies in genetically predisposed individuals initiates the autoimmune process
37 in a preclinical phase. The presence of anti-CCP antibodies could therefore be used as
38 prediction criteria for the development of RA in patients with UA.

1 Another more intuitive approach rather than an analytical one is to treat all UA pa-
2 tients with a relatively safe drug regardless of their post-test probability in the event of
3 new predictive tests. This would prevent that “false-negative” patients would not receive
4 aggressive therapy. It is however not (yet) clear how aggressive such a – at the same
5 time safe – therapy could be. It is unclear if such a therapy should be, for example, MTX,
6 corticosteroids or NSAIDs.

7 Current research is focusing on these treatments and on whether patients with UA will
8 benefit from early treatment with DMARDs to a similar extent as RA patients. In Leiden a
9 doubleblind placebo-controlled randomised trial (Probaat) with 110 patients who fulfill
10 the ACR 1958 criteria for probable RA and with a symptom duration of less than 2 years
11 is now underway. The aim of the study is to determine whether early treatment can pre-
12 vent progression into RA or even induce remission. The patients are being treated with
13 either placebo or MTX. After one year the medication will be tapered and then stopped.

14 The study ‘Stop Arthritis Very Early’ (SAVE) is another placebo-controlled study that
15 has just started and will try to modify the disease course of UA patients whose com-
16 plaints began less than 16 weeks earlier, with a single injection of methylprednisolone
17 i.m. Subgroup analyses may reveal whether all UA patients need to be treated or if only
18 a proportion of these patients will benefit from early treatment.

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22
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CHAPTER 3

Comparison of long term outcome of patients with rheumatoid arthritis presenting with undifferentiated arthritis or with rheumatoid arthritis: an observational cohort study

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1 Abstract**3 Background**

4 The outcome of undifferentiated arthritis (UA) ranges from remission to rheumatoid ar-
5 thritis (RA) fulfilling the American College of Rheumatology (ACR) classification criteria.

7 Objectives

8 To report the outcome of UA after 1-year of follow up and compare the disease course of
9 patients who presented with UA, but evolved into RA within 1 year (UA-RA group), with
10 that of patients who presented with RA fulfilling the ACR criteria (RA-RA group).

12 Methods

13 The diagnosis of 330 patients who presented with UA was recorded at 1-year. The UA-RA
14 and RA-RA groups were then followed up for 3 more years. Outcome measurements
15 were radiographic progression, disease activity, and functional capacity.

17 Results

18 From 330 patients who were diagnosed UA, 91 had evolved into RA at 1-year; 62 patients
19 had presented with RA. No significant differences were detected between the UA-RA
20 and RA-RA groups in median Sharp/van der Heijde score at baseline, radiographic
21 progression rates, disease activity, and functional capacity. However, significantly more
22 disease modifying antirheumatic drugs were prescribed in the RA-RA group.

24 Conclusion

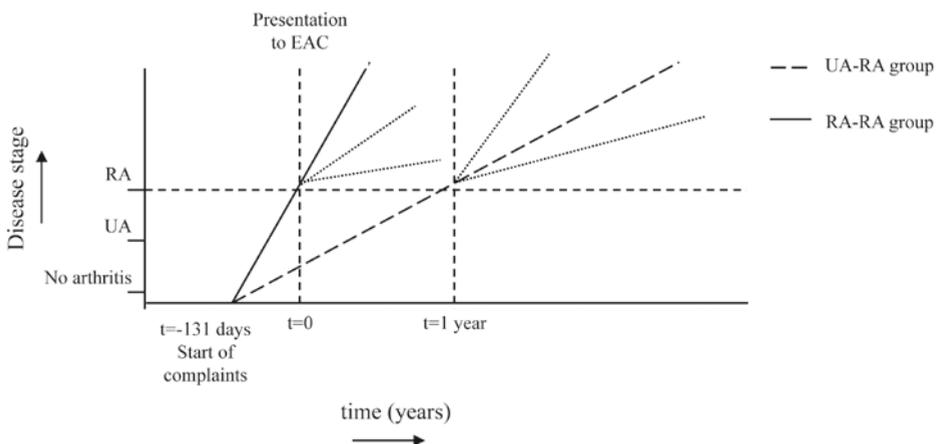
25 The disease outcome of patients who present with UA that evolves into RA within 1
26 year is the same as that of patients who present with RA as measured by radiographic
27 progression, disease activity, and functional capacity.

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1 Introduction

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3 In various studies of patients with recent onset arthritis, 6–55% of the patients who
4 presented with undifferentiated arthritis (UA) progressed into rheumatoid arthritis
5 (RA) according to the American College of Rheumatology (ACR) classification criteria or
6 according to the rheumatologist (1). Multivariate analysis of characteristics of these pa-
7 tients showed that the presence of antibodies to cyclic citrullinated peptide (anti-CCP),
8 polyarthritis, symmetric arthritis, and erosions on radiographs predict the development
9 of RA. Among these characteristics, anti-CCP has the highest specificity and positive
10 predictive value for RA (2). Thus, either by clinical or by serological data, clinicians will
11 be confronted with patients with UA who are likely to develop RA. The natural course of
12 patients who present with UA and subsequently develop RA is unknown compared with
13 patients who present with RA. In patients with RA many studies have demonstrated the
14 benefit of the early initiation of disease modifying antirheumatic drugs (DMARDs), re-
15 sulting in less disease activity and reduction of radiographic joint damage (3-5). Patients
16 included in these studies fulfilled the 1987 ACR classification criteria for RA (6). However,
17 the ACR classification criteria were not developed for the purpose of early diagnosis as is
18 illustrated by the fact that a minority of patients with inflammatory polyarthritis fulfilled
19 the ACR criteria at the first visit (7).

20 Although patients with RA benefit from early aggressive treatment (8), it is unknown
21 whether patients with UA whose disease will evolve into RA will also benefit from such
22 a treatment strategy. Hypothetically, if at presentation the duration of complaints is
23 similar, patients who present with UA might have a disease course that evolves more
24 slowly than that of patients who present with RA (**figure 1**). In the worst situation, the
25 “window of opportunity” for early treatment to induce long term disease modification
26



39 **Figure 1.** Hypothetical disease course of UA and RA

1 in the patients who presented with UA has been passed once the diagnosis RA is made,
2 and long term outcome will be comparable or worse than patients who were diagnosed
3 with RA at presentation and treated immediately.

4 Here, we describe the 1 year diagnosis and outcome of patients who presented with
5 UA, and compare the disease course of the patients with UA who had developed RA
6 within 1 year after presentation (UA-RA group) with that of patients who presented with
7 RA (RA-RA group). Radiographic progression was the major outcome measure; disease
8 activity and functional capacity were minor outcome measures.

10 **Patients and methods**

12 **Patients**

13 The Leiden Early Arthritis Clinic (EAC) was started in 1993. General practitioners were
14 asked to refer patients with suspected arthritis as soon as possible. Patients were
15 included in the EAC cohort if a rheumatologist confirmed the suspected arthritis and
16 the symptoms of arthritis did not exceed 2 years; for details of this cohort see Van der
17 Horst-Bruinsma *et al.*(9). Patient history, general physical examination, a painful and
18 swollen joint count, laboratory tests, questionnaires, and radiographic joint scores were
19 performed at inclusion and at the follow up visits. Based on the test results, a diagnosis
20 was recorded at the second visit, 2 weeks later, but could be revised during follow up.

22 **Study design**

23 From the EAC database (**figure 2**) patients diagnosed with UA at the second visit were
24 selected and their subsequent diagnosis was recorded at 1 year. UA comprised arthritis
25 of unknown cause and "probable RA" according to the 1958 ACR criteria (10). The group
26 of patients diagnosed with UA who evolved into RA fulfilling the 1987 ACR criteria within
27 1 year (UA-RA group) was followed up for 4 years. Because these patients had the op-
28 portunity to be treated with DMARDs as soon as the diagnosis was made, the long term
29 outcome was compared with that of a group of patients in whom RA was diagnosed at
30 the first and/or second visit and who still had RA after 1 year, and who were treated with
31 DMARDs as soon as RA was diagnosed (RA RA group).

33 **Outcome measurements**

34 Major outcome measurements were radiographic joint damage and calculated radio-
35 graphic progression rate. Radiographs of hands and feet were taken at inclusion in the
36 EAC and yearly thereafter. Radiographic scoring according to the Sharp/van der Heijde
37 method (11) was performed in random order by an experienced rheumatologist who
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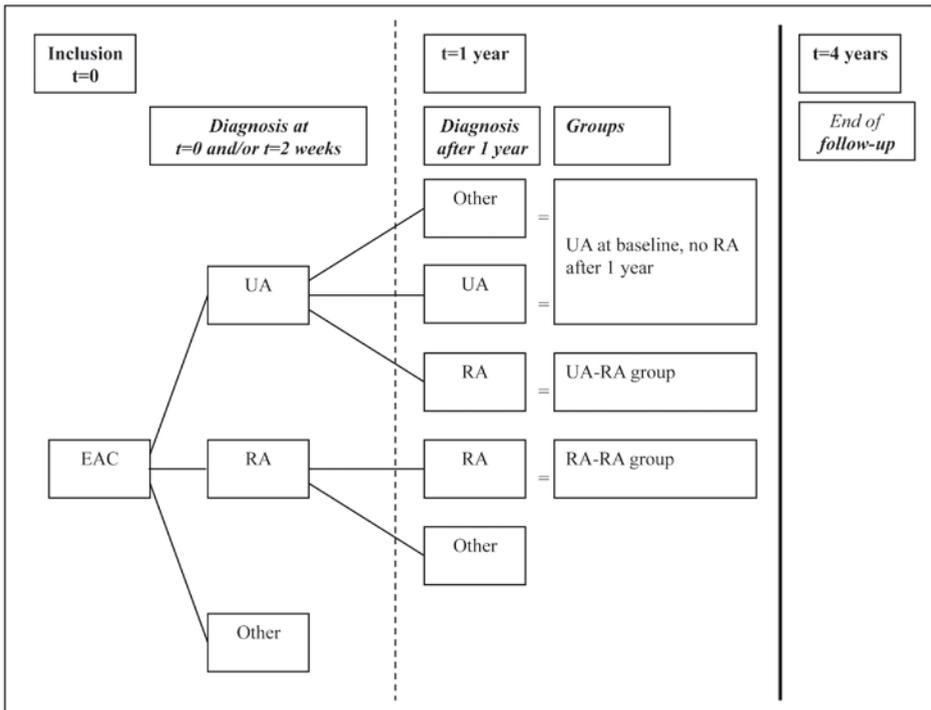


Figure 2. Flow diagram for definition of the groups

was unaware of the clinical data and the study questions. The intraclass correlation coefficient for the assessor's scoring was 0.95, as measured in 62 patients.

Minor outcome measurements were disease activity, functional capacity, erythrocyte sedimentation rate (ESR) in the 1st hour, and C reactive protein (CRP) concentration. Disease activity was calculated by a modified version of the disease activity score (DAS) (8):

$$(0.54 \times (\sqrt{\text{Ritchie score}}) + 0.065 \times (\text{swollen joint score}) + 0.33 \times \ln \text{ESR} + 0.224).$$

Functional capacity was measured by the Health Assessment Questionnaire (HAQ) (12).

Statistical analysis

Differences between patient characteristics were tested with the Mann-Whitney U test or the χ^2 test. For each patient separately, simple linear regression was used to fit the course of the radiographic progression in time. Progression rates were calculated for varying time intervals, assuming that radiographic damage progresses at a constant rate (13;14). The slopes of these regression lines estimate the average increase in Sharp/van der Heijde score per year. Differences between slopes of the groups of interest were tested with the Mann-Whitney U test.

To construct the radiographic progression as depicted in **figure 3**, the geometric mean of the Sharp/van der Heijde scores was estimated using a linear mixed model, in which account was taken of missing measurements (15). Differences in disease activity and functional capacity were compared with a linear mixed model. All tests were two tailed and p values ≤ 0.05 were considered significant.

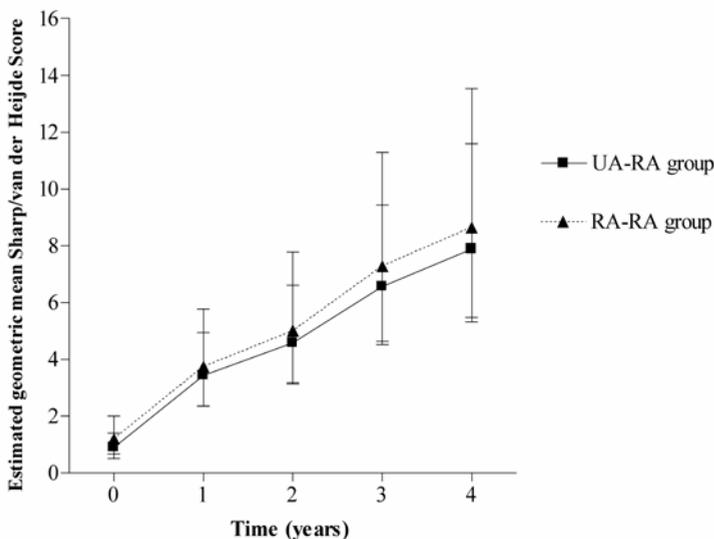


Figure 3. Estimated geometric mean Sharp/van der Heijde Score (95% confidence interval) of the UA-RA and RA-RA groups from baseline to 4 years

Results

Outcome of UA after 1 year

Between 1993 and 1999, 1064 patients were included in the EAC. At presentation, 330 patients were diagnosed with UA. Table 1 shows the diagnosis after 1 year of follow up of patients who had presented with UA and the numbers of patients who had ended follow up and their reasons for doing so. Ninety one patients had developed RA according to the 1987 ACR criteria (UA-RA group).

Patient characteristics at baseline

Table 2 shows the baseline characteristics of the UA-RA group, the RA-RA group, and the group of patients with UA who did not develop RA within 1 year. The UA-RA group differed from the RA-RA group in duration of morning stiffness, number of swollen joints,

Table 1. Diagnosis after 1 year of follow-up of patients who presented with UA and the numbers of patients who had ended EAC follow up at 1 year, and their main reason for doing so.

Diagnosis at 1 year	Patients No (%)	Patients who ended follow-up (n)	Main reason for end of follow-up No (%)
Arthritis of unknown cause	134 (40.6)	76	Remission 57 (75%)
Rheumatoid Arthritis	91 (27.6)	9	Death 3 (33%) Remission 3 (33%) Refusal 1 (11%) Other/unknown 2 (22%)
Probable RA	39 (11.8)	9	Remission 7 (78%)
Osteo-Arthrosis/Arthritis	11 (3.3)	10	End of treatment 7 (70%)
Psoriatic Arthritis	8 (2.4)	2	Remission 2 (100%)
Reactive Arthritis	6 (1.8)	6	Remission 6 (100%)
Crystal Induced Arthritis	4 (1.2)	3	EAC protocol 3 (100%)
Lyme Arthritis	4 (1.2)	2	Remission 2 (100%)
Paraneoplastic Arthritis	3 (0.9)	2	Remission 1 (50%) Life-threatening illness 1 (50%)
Systemic Lupus Erythematosus	2 (0.6)	0	
Palindromic RA	1 (0.3)	1	Move house 1 (100%)
Spondylarthropathy	1 (0.3)	1	EAC protocol 1 (100%)
Unknown cause	6 (1.8)	6	Remission 4 (67%)
Other causes	20 (6.1)	10	Remission 4 (40%)

Table 2. Patient characteristics at baseline of the RA-RA group, the UA-RA group, and patients with UA who did not develop RA within 1 year

Patient characteristics	RA-RA group (n=62)	p-value*	UA-RA group (n=91)	UA at presentation, no RA at 1 year (n=238)
Age (years)	53 (42-72)	0.683	55 (44-65)	46 (35-58)
Female patients	43 (69)	0.492	58 (64)	119 (50)
Duration of symptoms at first visit (days)	130 (61-278)	0.611	131 (73-230)	81 (20-164)
Duration of morning stiffness (min)	90 (45-150)	0.035	60 (18-120)	15 (0-60)
Number of swollen joints	7 (5-8)	<0.001	4 (2-6)	2 (1-3)
Modified DAS score	3.5 (3.1-4.0)	0.012	3.2 (2.7-3.7)	2.3 (1.9-2.8)
Rheumatoid factor positive	44 (71)	0.001	38 (42)	28 (12)
Anti-CCP antibodies	35/59 (59)	0.397	43/83 (52)	16 (8.2)
ESR (mm/1 st hour)	38 (20-56)	0.695	31 (19-53)	18 (9-37)
CRP (mg/l)	24 (9-41)	0.414	17 (8-46)	10 (4-26)
HAQ score	1.0 (0.6-1.4)	0.184	0.8 (0.4-1.2)	0.6 (0.1-1.0)
Erosive disease at baseline (hands and feet radiographs)	18/60 (30)	1.000	26/88 (30)	20 (13)
Sharp/van der Heijde score	1 (0-4)	0.281	0 (0-3)	0 (0-0)

*p Value of RA-RA group versus UA-RA group.

Results are shown as number (%) or median (interquartile range).

1 modified DAS, and percentage of patients who were rheumatoid factor (RF) positive.
 2 These differences reflect the ACR criteria for RA.

3 The patients with UA who did not develop RA within 1 year were significantly different
 4 at baseline from both the UA-RA group and the RA-RA group in all characteristics.

6 Radiographic joint damage

7 No significant differences were detected between the UA-RA and RA-RA groups for
 8 median Sharp/van der Heijde score (**table 3, figure 3**). At 4 years 75% of all patients in
 9 both the UA-RA group (77%) and the RA-RA group (73%) had erosive disease compared
 10 with 30% at baseline.

11 **Table 3.** Sharp/van der Heijde scores and radiographic progression rates (increase in score per year) in the
 12 UA-RA and RA-RA groups

	UA-RA group (n=91)		RA-RA group (n=62)	
	n	Median (IQR)	n	Median (IQR)
<i>Sharp/van der Heijde score</i>				
Baseline	88	0 (0-3)	60	1 (0-4)
1 year	79	4 (0-13)	56	5 (0-18)
2 years	71	8 (0-17)	48	5 (1-27)
3 years	66	10 (2-20)	45	11 (2-26)
4 years	53	9 (2-30)	41	15 (4-27)
<i>Radiographic progression rate</i>				
Slope 0-1 year*		2 (0-8)		2 (0-10)
Slope 0-2 years*		3 (0-8)		1 (0-8)
Slope 0-4 years*		2 (0-7)		2 (0-4)
Slope 1-4 years*		2 (0-8)		1 (-1-3)
Slope 2-4 years*		2 (-1-8)		2 (0-5)

29 *p>0.05 in the UA-RA group versus the RA-RA group.

30 n is the number of patients with available data.

32 Disease activity and functional capacity

33 **Figures 4A-D** show the mean DAS, HAQ, ESR, and CRP of the two groups. During follow
 34 up no significant differences were seen.

36 DMARDs

37 **Table 4** shows the use of DMARDs. At all time points significantly fewer DMARDs were
 38 prescribed in the UA-RA group than in the RA-RA group. Frequently prescribed DMARDs
 39 were hydroxychloroquine, sulphasalazine, and methotrexate or a combination of these.

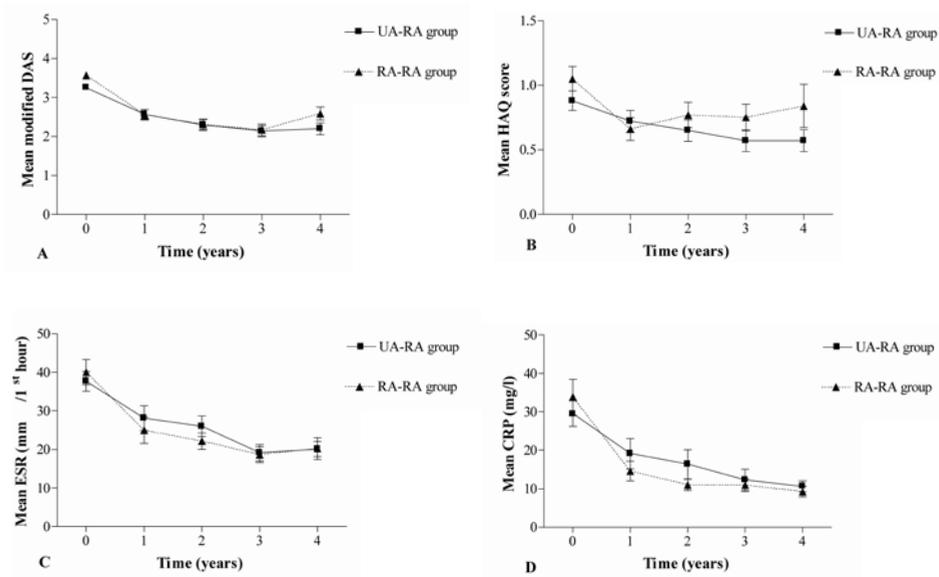


Figure 4. Secondary outcome values of the UA-RA and RA-RA groups from baseline to 4 years. (A) Modified Disease Activity Score; (B) Health Assessment Questionnaire score; (C) Erythrocyte Sedimentation Rate in mm/1st h; (D) C reactive protein concentration. Mean values (SEM).

Table 4. Number (%) of patients receiving prescribed DMARDs at 3 months, 1, 2, 3 and 4 years in the UA-RA and RA-RA groups

Time points	UA-RA group (n=91)	RA-RA group (n=62)	p Value
3 months	42 (46)	62 (100)	<0.001
1 year	62 (68)	58 (94)	<0.001
2 years	63 (69)	56 (90)	<0.001
3 years	60 (66)	49 (79)	0.04
4 years	52 (57)	48 (77)	0.003

Prednisolone was also prescribed as single drug or combination therapy. Gold, azathioprine, and ciclosporin were prescribed in a minority of patients, and biological agents were not prescribed at all.

In the UA-RA group, 16/91 patients never received DMARDs during the follow up period of 4 years. Of these 16 patients, 11 were RF negative and in 10 patients erosive disease was seen during follow up.

Dropouts

Follow up of 3 - 4 years was available for 124 patients. Twenty nine patients were lost to follow up (dropouts). In the UA-RA group five patients were in remission, two refused follow up, five had died, one discontinued because of a concomitant illness, and in six

1 patients the cause was unclear. In the RA-RA group four patients were in remission, two
 2 refused follow up, one had died, one discontinued because of a concomitant illness,
 3 and in two patients the cause remained unclear. **Table 5** compares the baseline char-
 4 acteristics of the dropouts with those of patients with 4 year follow up (completers).
 5 Only CRP in the UA-RA group was significantly higher in the dropout group than for the
 6 completers

7
 8 **Table 5.** Baseline characteristics of dropouts (before or at 3 years of follow-up) versus completers

Patient characteristics	UA-RA group			RA-RA group		
	Dropouts (n=19)	Completers (n=72)	p Value	Dropouts (n=10)	Completers (n=52)	p Value
Age (years)	64 (46-75)	55 (44-63)	0.099	57(36-85)	53 (46-69)	0.745
Female patients	57	65	0.598	60	71	0.479
Duration of symptoms at first visit (days)	102 (61-209)	144 (78-250)	0.381	159 (63-275)	126 (59-290)	0.851
Duration of morning stiffness (min)	60 (30-180)	60 (15-120)	0.341	75 (41-135)	90 (45-180)	0.875
Number of swollen joints	5 (3-8)	4 (2-5)	0.087	7 (4-8)	7 (5-8)	0.658
Modified DAS score	3.3 (2.8-4.2)	3.1 (2.6-3.6)	0.218	3.5 (2.9-4.7)	3.5 (3.1-4.0)	0.626
RF- positive	26	47	0.127	60	73	0.457
anti-CCP antibodies	42	55	0.435	50	61	0.725
ESR (mm/1 st hour)	37 (25-65)	30 (18-50)	0.091	46 (25-61)	35 (19-57)	0.410
CRP (mg/l)	57 (13-74)	11 (7-32)	0.002	19 (9-44)	27 (9-42)	0.984
HAQ score	1.0 (0.12-1.37)	0.75 (0.5-1.12)	0.852	1.31 (0.65-1.75)	1.0 (0.5-1.37)	0.382
Erosive disease (hands and feet radiographs)	33	29	0.695	44	28	0.309

25 Results are shown as number or median (interquartile range)

27 Discussion

28 Here, the hypothesis was tested whether patients with UA who developed RA within
 29 1 year have a different disease course than those who presented to the Leiden EAC
 30 with RA fulfilling the ACR criteria (6). Of the 330 patients who initially presented with
 31 UA, 91 (28%) had developed RA within 1 year. Although the patients with UA might
 32 hypothetically have evolved more slowly towards RA, joint damage, disease activity, and
 33 functional capacity were the same in both groups over a follow up period of 4 years.
 34

35 UA is a commonly encountered problem in daily practice. In one third of patients with
 36 recent onset arthritis it is not possible to come to a definite diagnosis at presentation.
 37 Overall, 63% of the patients at the Leiden EAC had a self limiting disease course and 28%
 38 progressed from UA to RA. Other studies show that 6 - 55% of patients with UA will even-
 39

1 tually evolve into RA (1). These percentages are dependent on the different inclusion
2 criteria, recruitment procedures, and disease criteria. For example, low percentages are
3 found in RF negative patients with monarthritis (16), and higher percentages are found
4 in inception cohorts where patients with polyarthritis are diagnosed with RA, regardless
5 of fulfilment of the ACR criteria (17).

6 Despite the fact that many studies have tried to describe the natural history of UA, the
7 long term outcome of the subgroup of patients with UA who develop RA has never been
8 described. As can be expected, the UA-RA and RA-RA groups differed at presentation in
9 duration of morning stiffness, number of swollen joints, modified DAS, and percentage
10 of RF positive patients, because these variables are directly or indirectly part of the 1987
11 ACR classification criteria for RA. The presence of anti-CCP was equal in both groups and
12 is similar to previous studies (18). The group of patients with UA who did not develop RA
13 within 1 year differed significantly in all patient characteristics at baseline from the other
14 two groups. This is a very heterogeneous group considering the diagnosis after 1 year,
15 but all these patients were diagnosed with UA at baseline.

16 Median symptom duration is about the same in both groups. However, given the large
17 variation in symptom duration some misclassification of patients might have occurred.
18 Theoretically, patients with UA who presented with short symptom duration might have
19 belonged to the RA-RA group, and patients with RA with very long symptom duration
20 might have had the chance of being included in the UA-RA group. However, analysis of
21 subgroups with different intervals of symptom duration did not show an effect on joint
22 damage.

23 In the dropout analysis, only CRP in the UA-RA group was significantly higher in the
24 dropout group than for the completers. These data argue against the possibility that
25 the UA-RA group equals the RA-RA group because the dropouts in the UA-RA group
26 were the patients who presented with a milder disease, and only the patients with more
27 aggressive disease completed the follow up.

28 At all time points, the UA-RA group received fewer DMARDs than the RA-RA group. In
29 this observational study, this might reflect a historical development in DMARD prescrip-
30 tion. It also might be a confounding by indication, as clinicians are inclined to prescribe
31 DMARDs to patients who they expect to have a more destructive disease course. Then,
32 patients in the RA-RA group might have a more aggressive disease course that is modi-
33 fied by DMARDs. However, the power to detect an effect of the presence or absence
34 of DMARD use on radiographic progression was too low given the small number of
35 patients with RA who did not use DMARDs (n=16). In the UA-RA group, 16/91 patients
36 did not receive DMARDs during the 4 year follow up. Although 11 of these patients were
37 RF negative, 10 had erosive disease. However, with a median radiographic progression
38 of one point a year, this subgroup had less progressive disease than the group treated
39

1 with DMARDs. A randomised clinical trial with DMARDs in patients with UA would be the
2 most appropriate study design to overcome this issue.

3 The overall outcome of UA poses challenging treatment goals. Patients with UA who
4 will go into remission will need treatment aimed at reducing symptoms, whereas pa-
5 tients prone to progress to RA will need treatment to improve their long term outcome
6 as well. Furthermore, there is a need for new criteria to identify patients with UA that
7 will evolve into RA, as the current ACR criteria for RA are not suitable for this purpose.
8 This study implies that the long term outcome of patients who present with UA which
9 evolves into RA is the same as that of patients who present with RA. New serological
10 tests combined with clinical judgment will help to determine which patients with UA
11 are most likely to develop RA. Randomised clinical trials in such patients with UA are
12 currently under way to test whether this group of patients may benefit from very early
13 DMARD treatment.

14 15 **Acknowledgements**

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17 Netherlands Organisation for Scientific Research (NWO, grant No 920-03-259).
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CHAPTER 4

Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis. A double-blind, randomized, placebo-controlled trial

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1 Abstract**3 Objective**

4 To determine whether patients with undifferentiated arthritis (UA; inflammatory, non-
5 traumatic arthritis that cannot be diagnosed using current classification criteria) benefit
6 from treatment with methotrexate (MTX).

8 Methods

9 The PRObable rheumatoid arthritis: Methotrexate versus Placebo Treatment (PROMPT)
10 study was a double-blind, placebo-controlled, randomized, multicenter trial involving
11 110 patients with UA who fulfilled the American College of Rheumatology (ACR) 1958
12 criteria for probable RA. Treatment started with MTX (15 mg/week) or placebo tablets,
13 and every 3 months the dosage was increased if the Disease Activity Score was >2.4.
14 After 12 months, the study medication was tapered and discontinued. Patients were
15 followed up for 30 months. When a patient fulfilled the ACR criteria for RA (primary
16 end point), the study medication was changed to MTX. Joint damage was scored on
17 radiographs of the hands and feet.

19 Results

20 In 22 of the 55 patients (40%) in the MTX group, UA progressed to RA compared with 29
21 of 55 patients (53%) in the placebo group. However, in the MTX group, patients fulfilled
22 the ACR criteria for RA at a later time point than in the placebo group ($P = 0.04$), and
23 fewer patients showed radiographic progression over 18 months ($P = 0.046$).

25 Conclusion

26 This study provides evidence for the efficacy of MTX treatment in postponing the
27 diagnosis of RA, as defined by the ACR 1987 criteria, and retarding radiographic joint
28 damage in UA patients.

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1 Introduction

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3 For a number of autoimmune diseases, such as diabetes mellitus, it has been sug-
4 gested that a critical period exists during which intervention may reverse the disease
5 process (1). For rheumatoid arthritis (RA), such a window of opportunity may also exist,
6 because laboratory abnormalities, such as antibodies against cyclic citrullinated peptide
7 (anti-CCP), can occur years before disease onset (2). In previous studies of patients with
8 undifferentiated arthritis (UA), defined as an inflammatory arthritis in which no definitive
9 diagnosis can be made (3), it was observed that the presence of anti-CCP, with an odds
10 ratio of 38, is an important predictor of the development of RA (4). Of all the patients who
11 present with UA, depending on the study population, 6-55% develop RA within 1 year (5).

12 Current treatment of UA patients mainly consists of nonsteroidal antiinflammatory
13 drugs (NSAIDs), and treatment with disease-modifying antirheumatic drugs (DMARDs) is
14 not initiated until the disease has progressed to RA (3). Once patients fulfill the American
15 College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987
16 criteria for RA (6), early initiation of DMARD treatment results in less disease activity,
17 reduction of radiographic joint damage, and maintenance of function, as compared
18 with delayed treatment (7-9). Consequently, it is hypothesized that DMARD treatment
19 that is started as early as possible in UA patients may alter disease progression and may
20 prevent the development of RA.

21 Therefore, we designed a double-blind, placebo-controlled, randomized clinical
22 trial to compare 2 treatment strategies. The immediate treatment strategy consisted of
23 methotrexate (MTX) for a course of 1 year followed by tapering the amount of treatment
24 if UA had not evolved into RA (as defined by fulfillment of the ACR classification criteria).
25 The control group received conventional treatment with NSAIDs, and MTX therapy was
26 initiated only if the patients fulfilled the ACR criteria for RA (the primary end point).
27 The primary outcomes of the PRObable RA: Methotrexate versus Placebo Treatment
28 (PROMPT) study were diagnosis at the end of the study and progression of radiographic
29 joint damage.

30 31 Patients and methods

32 33 Study setting and design

34 The PROMPT study was a prospective double-blind, randomized, placebo-controlled,
35 multicenter trial involving 110 patients. Randomization was performed by the pharma-
36 cist. The study was conducted between March 2001 and January 2006 in 4 hospitals in
37 Leiden, The Hague, and Delft, The Netherlands. The study was approved by the medical
38 ethics committee of the participating hospitals. Patients started with 6 tablets, each
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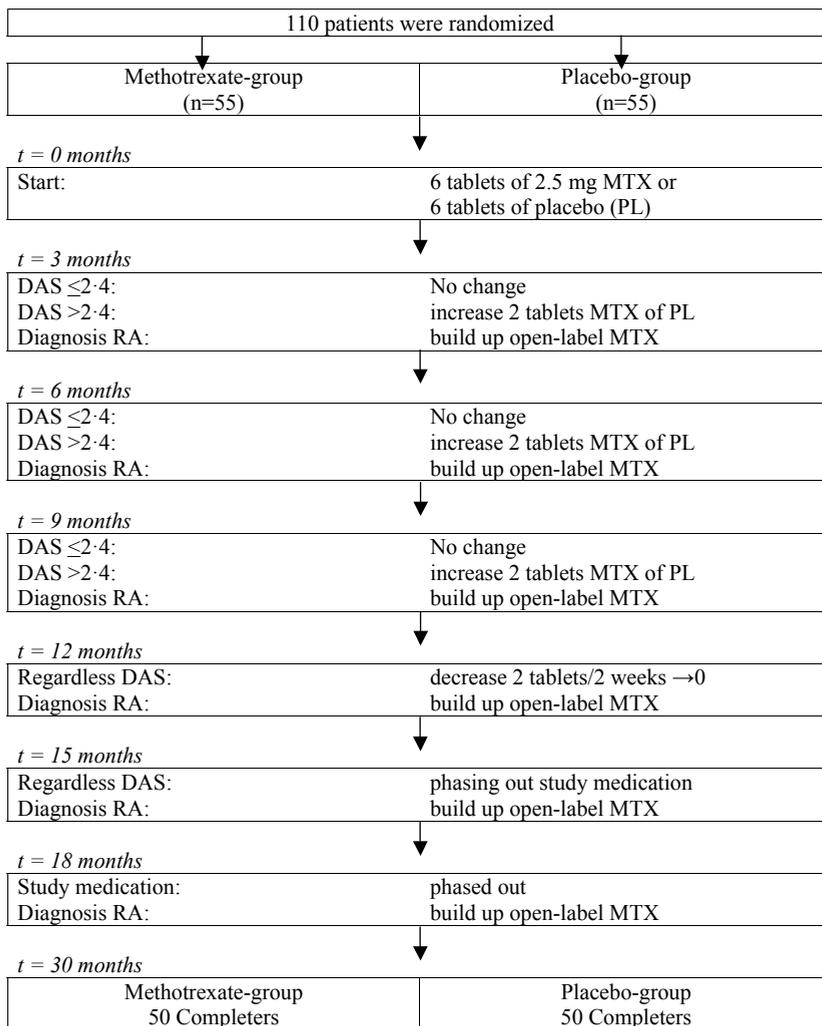


Figure 1. Overview of treatment strategy. Patients received open-label methotrexate (MTX) if rheumatoid arthritis (RA; n=49) or psoriatic arthritis with erosive disease (n=2) was diagnosed. PL = placebo; DAS = Disease Activity Score.

containing either 2.5 mg MTX or placebo. Every 3 months, the medication was increased by 2 tablets if the Disease Activity Score (DAS) was > 2.4 (10), to a maximum of 12 tablets or 30 mg of MTX (**Figure 1**). The DAS was calculated using a tender joint count (Ritchie Articular Index; RAI), the erythrocyte sedimentation rate (ESR), and a visual analog scale (VAS) for general health status, according to the following formula:

$$\text{DAS} = 0.54 \times \sqrt{(\text{RAI})} + 0.065 \times (\text{swollen joint count}) + 0.33 \times \ln(\text{ESR}) + 0.0072 \times (\text{VAS general health})$$

1
2 Trained research nurses calculated the tender and swollen joint scores. To minimize the
3 side effects of MTX, all patients, including those in the placebo group, received folic acid
4 daily (1 mg) or weekly (5 mg). In both groups, patients were allowed to take NSAIDs,
5 but no other immunosuppressive therapies, including steroids, were allowed. In cases of
6 side effects that might be related to MTX, the treatment was adjusted.

7 If a patient reached the primary end point during followup, defined as fulfilling the
8 ACR 1987 RA classification criteria, it was considered unethical to continue with study
9 medication (possibly placebo), and the treatment was initially continued by building
10 up open-label MTX to the same amount as in the study medication scheme. After 12
11 months, the study medication was decreased by 2 tablets every 4 weeks until it reached
12 a level of 0 in the patients who did not reach the primary end point. At study inclusion
13 and at 3, 6, 9, 12, and 18 months thereafter, a tender and swollen joint count, a Health
14 Assessment Questionnaire, and a VAS for general health were obtained (11;12). Every 6
15 months, radiographs of hands and feet were obtained. At 30 months, the diagnosis was
16 recorded.

17 18 **Participants**

19 Eligible patients attended the rheumatology outpatient clinic of the participating hos-
20 pitals, had symptoms of arthritis that did not exceed 2 years in duration, were 18 years
21 of age or older, and were diagnosed as having UA (i.e., did not fulfill classification criteria
22 for any rheumatologic disorder). Given the lack of criteria for UA, patients had to fulfill
23 the ACR 1958 criteria for probable RA (13). One patient with psoriasis was also included
24 because the small joints of the hands and feet were involved. Exclusion criteria were RA
25 (according to the ACR 1987 criteria), impaired kidney or liver function, alcoholism, bone
26 marrow insufficiency, pregnancy or the desire to become pregnant within 21 months
27 from inclusion in the study, and DMARD use in the past. All patients provided written
28 informed consent.

29 30 **Outcome**

31 The diagnosis at the end of the study and the radiographic progression were prespeci-
32 fied primary outcomes. After 30 months, a diagnosis of RA, UA, remission, or other was
33 recorded. Remission was defined as no clinical symptoms of arthritis according to the
34 patient's rheumatologists and no DMARD use in the preceding year.

35 Radiographic damage was graded by 2 experienced readers (JvA and HvD) using the
36 Sharp/van der Heijde scoring method (SHS), with the radiographs in chronological order
37 and patient identity masked (14;15). The interobserver intraclass correlation coefficient
38 (ICC) was 0.898. The intraobserver ICCs for both readers were 0.990 and 0.993, as mea-
39 sured in 20 patients. The smallest detectable change (SDC) was 3.02 (16). Prespecified

1 secondary outcomes were changes in disease activity represented by the ESR and the
2 DAS.

3 For a post hoc analysis of the outcomes according to anti-CCP status, the presence
4 of anti-CCP was measured at the end of the study in baseline serum samples from all
5 patients, before decoding the treatment arms.

6 7 **Statistical analysis**

8 Using historical data from the Leiden Early Arthritis Clinic (EAC), 40% of the patients
9 with UA were expected to develop RA within 1 year (4). It was estimated that immediate
10 treatment with MTX would result in <20% of the patients with UA developing RA. A
11 sample size of 46 patients per treatment group was required in order to attain a power
12 of 80% to detect a significant difference between groups with a *P* value of 0.05. To allow
13 for dropouts, 110 patients were included in the study.

14 Demographic and baseline characteristics, changes in the DAS and ESR, and radio-
15 graphic progression in the 2 treatment groups were compared using the chi-square test,
16 Student's 2-tailed t-test, or the Mann-Whitney U test. Because MTX reduces radiographic
17 joint damage in RA and the SHS method does not allow for healing, radiographic pro-
18 gression was tested 1-sided (15;17). Differences in the development of RA during the
19 study were determined using a Kaplan-Meier curve with a log rank test. The Cox propor-
20 tional hazards model yielded the hazard ratios and 95% confidence intervals. *P* values
21 less than 0.05 were considered statistically significant.

22

23

24 **Results**

25

26 **Characteristics of the study patients**

27 The demographic and clinical characteristics of the patients at baseline are shown in
28 **Table 1**. To allow for assessment of the external validity of this trial, the baseline char-
29 acteristics of the total group of patients in the PROMPT study were compared with a
30 control group of UA patients who were included in the Leiden EAC study between 1993
31 and 1999 (3). In the PROMPT study group, the duration of symptoms was longer and the
32 proportion of patients who were rheumatoid factor (RF)-positive was higher than in the
33 EAC group. The ESR and C-reactive protein (CRP) levels were lower in the PROMPT group
34 than in the EAC group.

35

36 **Diagnosis at the end of the study**

37 **Figure 1** shows the randomization and treatment strategy. After 30 months, in 22 of
38 55 of the patients in the MTX group (40%) versus 29 of 55 in the placebo group (53%)
39 UA had eventually progressed to RA. However, in the placebo group, all patients whose

Table 1. Demographic and clinical characteristics at baseline of members of the PROMPT study and of the UA patients in the Leiden EAC study*

	MTX group n=55	Placebo group n=55	EAC group n=330
Age, years	51 (42-60)	51 (42-56)	48 (36-61)
Female, no. (%)	35 (64)	38 (69)	177 (54)†
Duration of symptoms at first visit, days	312 (195-507)	263 (169-432)	92 (31-186) †
Duration of morning stiffness, minutes	30 (10-60)	30 (10-60)	30 (0-60)
No. of swollen joints	3 (2-5)	2 (2-4)	2 (1-4) †
3-variable DAS	2.7 (2.1-3.1)	2.4 (2.0-3.0)	2.5 (2.0-3.1)
4-variable DAS	2.7 (2.2-2.7)	2.5 (2.0-3.0)	
RF positive, no. (%)	20 (36)	19 (35)	66 (21) †
Anti-CCP positive, no. (%)	12 (22)	15 (27)	55 (20)
ESR, mm/hour	12 (5-24)	11 (5-25)	22 (10-40) †
CRP, mg/liter	5 (3-11)	5 (3-9)	11 (5-31) †
HAQ score	0.75 (0.38-1.13)	0.75 (0.25-1.13)	0.62 (0.25-1.12)
Patients with erosive disease, no. (%)	2 (4)	3 (6)	37 (16) †
Sharp/van der Heijde score	0.5 (0-2.5)	1 (0-3.0)	0 (0-1.0) †

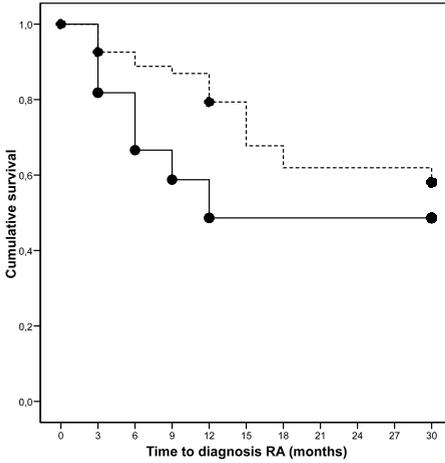
* Except where indicated otherwise, values are the median (interquartile range). PROMPT = PROBable rheumatoid arthritis: Methotrexate versus Placebo Treatment; UA = undifferentiated arthritis; EAC = Early Arthritis Clinic; MTX = methotrexate; DAS = Disease Activity Score; RF = rheumatoid factor; anti-CCP = anti-citrullinated peptide antibody; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; HAQ = Health Assessment Questionnaire.

† $P \leq 0.05$ versus the PROMPT group.

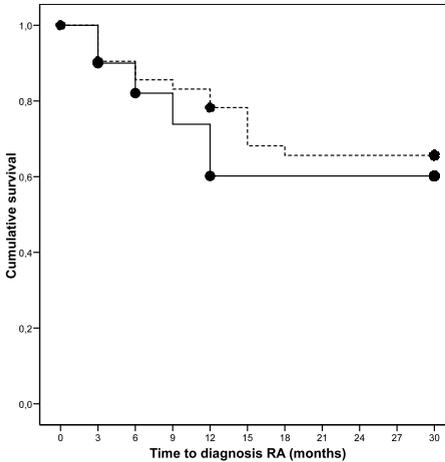
disease had progressed to RA fulfilled the ACR criteria within 1 year, versus only one-half of the RA patients in the MTX group ($P = 0.04$) (**Figure 2A**). The other half of the RA patients in the MTX group reached the diagnosis during or after tapering of the study medication. After 30 months, a similar number of patients achieved remission in both treatment groups: 15 in the MTX group and 13 in the placebo group (**Table 2**).

Radiographic progression

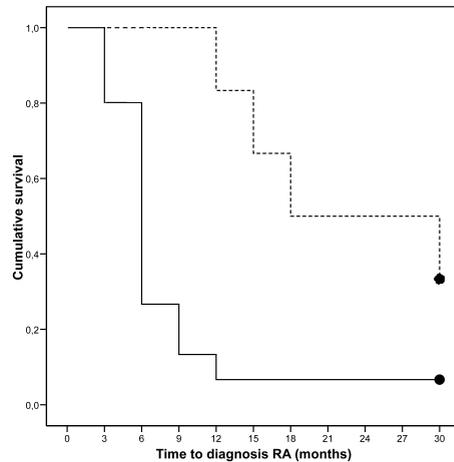
The distribution of the radiographic progression over 18 months is shown in **Figure 3A**. In both groups, 51 patients had completed radiographic followup. After 18 months, the majority of patients had no radiographic progression at all: 73% in the placebo group and 88% in the MTX group. However, 6 patients in the MTX group versus 14 patients in the placebo group showed radiographic progression above the SDC ($P = 0.046$). Individual progression measured only in patients with erosions was significantly lower in the MTX group versus the placebo group ($P = 0.035$).



A



C



B

Figure 2. Kaplan-Meier survival analysis for the diagnosis of rheumatoid arthritis (RA). The methotrexate (MTX) group is indicated by the broken line, the placebo group is indicated by the solid line, and drop-outs are indicated by circles. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) indicate the risk of developing RA during the study in the placebo group versus the MTX group. (A) Total group (n=110) (HR 1.7 [95% CI 0.99-3.01], $P=0.04$). (B) Members of the subgroup positive for antibodies against cyclic citrullinated peptide (anti-CCP) (n=27) (HR 4.9 [95% CI 1.88-12.79], $P<0.001$). (C) Members of the subgroup negative for anti-CCP (n=83) (HR 1.3 [95% CI 0.61-2.63], $P=0.51$).

Subgroup analysis

In the anti-CCP-positive subgroup treated with placebo, UA in 14 of the 15 patients (93%) progressed to RA and did so at an earlier time point than that in 8 of the 12 patients (67%) in the MTX group ($P < 0.001$) (**Table 2 and Figure 2B**). In contrast, in the anti-CCP-negative subgroup no differences in outcome at 30 months were seen (**Figure**

Table 2. Diagnosis at 30 months, by subgroup*

Group (n)	RA	UA	UA in remission	Other	Lost to follow up
Total					
MTX (55)	22	10	15	3 (2 osteoarthritis, 1 autoimmune hepatitis)	5
Placebo (55)	29	4	13	4 (3 osteoarthritis, 1 diabetic arthropathy)	5
Anti-CCP-positive					
MTX (12)	8	2	2	0	0
Placebo (15)	14	0	1	0	0
Anti-CCP-negative					
MTX (43)	14	8	13	3 (2 osteoarthritis, 1 autoimmune hepatitis)	5
Placebo (40)	15	4	12	4 (3 osteoarthritis, 1 diabetic arthropathy)	5

* RA = rheumatoid arthritis; UA = undifferentiated arthritis; MTX = methotrexate; anti-CCP= anti-cyclic citrullinated peptide antibody

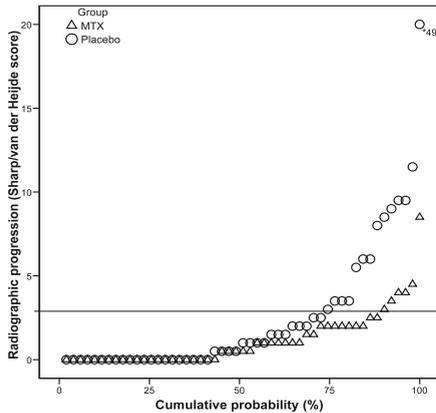
2C). Similar effects on radiographic progression were observed (**Figures 3B and C**). In the anti-CCP-negative group, no MTX effect could be detected, whereas in the anti-CCP-positive group, the progression was slowed down significantly ($P = 0.03$).

Subgroup analysis for the presence of RF showed considerable overlap with the presence of anti-CCP: 26 of 39 RF-positive patients (67%) were anti-CCP positive, whereas 60 of 61 RF-negative patients (98%) were anti-CCP negative. In the placebo group, UA in 13 of the 19 RF-positive patients (68%) progressed to RA and did so at an earlier time point than did 11 of the 20 RF-positive patients (55%) in the MTX group ($P = 0.036$). In contrast, in the RF-negative subgroup, no differences in outcome at 30 months were seen ($P = 0.403$). With regard to radiographic progression, RF-positive patients in the placebo group showed a trend for more radiographic progression than those in the MTX group (data not shown).

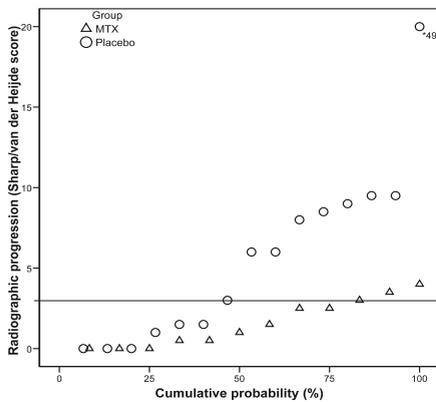
Subgroup analysis of the development of RA over time for autoantibody-positive (anti-CCP-positive or RF-positive) patients weakened the significance as compared with analysis of the anti-CCP-positive patients alone ($P = 0.024$). Of the 27 anti-CCP-positive patients, only 1 was RF negative. This patient received placebo and was diagnosed as having RA 6 months after study inclusion. The outcome of the 26 patients who were anti-CCP-positive and RF-positive is shown in **Table 2**.

Cross-sectional followup

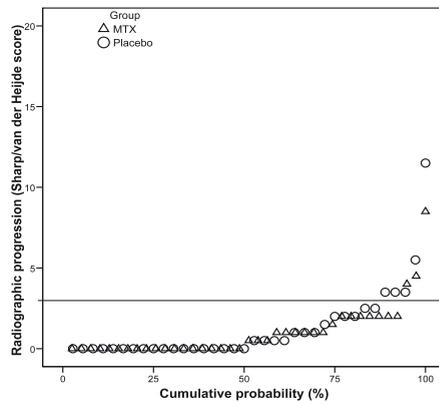
At the time of submission of this article, further followup data were not available for all patients due to different followup periods. However, we can report on 5 of 27 patients in the anti-CCP-positive subgroup (18%) who did not develop RA after 30 months (**Table 2**). The 3 patients who were in remission at 30 months (1 in the placebo group and 2 in the MTX group) were still in remission without DMARD treatment 4 years after study inclusion. However, the 2 patients with persistent UA (both from the MTX group) restarted MTX treatment after 30 months, 1 because of recurrent symptoms and, ulti-



A



B



C

Figure 3. Occurrence of radiographic progression. The presence or absence of radiographic progression (Sharp/van der Heijde score) at 18 months in 102 patients who completed radiographic followup was calculated. Each symbol represents 1 patient. Horizontal lines represent the smallest detectable difference (indicating radiographic progression). A, Total group ($n = 102$). B, Patients positive for antibodies against cyclic citrullinated peptide (anti-CCP) ($n = 27$). C, Patients negative for anti-CCP ($n = 75$). For methotrexate (MTX)-treated versus placebo-treated patients, $P = 0.15$ in the total group, $P = 0.03$ in the anti-CCP-positive patients, and $P = 0.46$ in anti-CCP-negative patients.

mately, arthritis shortly after withdrawal of study medication and 1 because of recurrent arthritis after a 6-month remission period. The patients continued to receive MTX at the time of submission of this article. As a result, although only 1 fulfilled the ACR criteria for RA, it can be argued that they both have RA, given the recurrent arthritis symptoms that require DMARDs. Taking these data into account, 10 of 12 anti-CCP-positive patients (83%) in the MTX group eventually developed RA and 2 of 12 patients (17%) achieved sustained remission, emphasizing that MTX postponed, but did not prevent, RA.

1 Disease activity

2 After 3 months, the mean decrease in DAS (from 2.7 to 2.3) and ESR (from 17 mm/hour
3 to 13 mm/hour) values in the MTX group differed significantly from the mean change in
4 DAS (from 2.5 to 2.5) and ESR (from 15 mm/hour to 16 mm/hour) in the placebo group
5 ($P = 0.01$ and $P = 0.02$, respectively). However, subgroup analysis showed that in the
6 anti-CCP-positive patients, the mean DAS decreased (from 2.8 to 1.9) in the MTX group
7 and increased in the placebo group (from 2.6 to 3.1) ($P < 0.001$). However, in the anti-
8 CCP-negative patients, the mean DAS decreased irrespective of treatment group (from
9 2.7 to 2.4 versus from 2.5 to 2.3 in the MTX and placebo groups, respectively; $P = 0.62$).

10 After 12 months, study medication was decreased regardless of the DAS. In the MTX
11 group, 8 of 40 patients who still received study medication had a DAS >2.4 at 12 months
12 and started phasing out the study medication; the UA in 2 of these 8 patients later pro-
13 gressed to RA. Of the 32 patients with a DAS ≤ 2.4 , the UA in 9 of them progressed to RA.
14 Of the anti-CCP-positive patients in the MTX group, only 1 of the 10 who still received
15 study medication at 12 months had a DAS >2.4 and later developed RA. Of the 9 patients
16 with a DAS ≤ 2.4 , 5 developed RA, 2 had UA without fulfilling the ACR criteria for RA at 30
17 months, and 2 went into remission.

18 Toxicity

19 Adverse events were recorded during the first 18 months, the intervention period of
20 the study. Of the patients who were taking study medication, 26 of 55 patients (47%) in
21 the MTX group and 18 of 55 patients (33%) in the placebo group experienced ≥ 1 (seri-
22 ous) adverse event ($P = 0.173$). While taking the study medication, a total of 44 adverse
23 events and 5 serious adverse events occurred in the MTX group versus a total of 25
24 adverse events and 4 serious adverse events in the placebo group (**Table 3**).

25 When patients fulfilled the ACR 1987 RA classification criteria during followup, they
26 were switched to open-label MTX. If MTX produced undesirable adverse events or was
27 ineffective, other DMARDs were prescribed. The 20 patients who developed RA in the
28 former MTX group in the first 18 months were subsequently treated with MTX (19 pa-
29 tients), sulfasalazine (1 patient), hydroxychloroquine (3 patients), and/or leflunomide (1
30 patient). Adverse events (12 adverse events and 3 serious adverse events) were reported
31 in 7 of 20 patients while they were treated with these DMARDs. In the former placebo
32 group, the 29 patients who developed RA in the first 18 months were treated with MTX
33 (29 patients), sulfasalazine (2 patients), hydroxychloroquine (5 patients), leflunomide (1
34 patient), infliximab (2 patients), etanercept (1 patient), and/or adalimumab (2 patients).
35 Adverse events (39 adverse events and 3 serious adverse events) were reported in 18 of
36 29 patients while they were treated with these DMARDs.

Table 3. Adverse events and serious adverse events during the use of the study medication and during the use of DMARDs after RA diagnosis, by treatment group*

	During use of study medication		During use of DMARDs after diagnosis RA	
	MTX	Placebo	Former MTX	Former Placebo
All adverse events				
Gastrointestinal	11	6	1	12
Dermal / mucosal	9	7	2	5
Neurologic	3	5	5	4
Cardiologic	3	-	-	2
Pulmonary	3	1	3	1
Haematologic	1	1	-	-
Ophthalmologic	3	-	1	2
Elevated serum liver enzyme levels	6	1	-	4
Other				
Tiredness	1	1	-	2
Giant cell tumor	1	-	-	-
Rhinitis	1	-	-	-
Not feeling well	2	-	-	1
Fracture	-	1	-	-
Hair loss	-	1	-	3
Synovectomy	-	1	-	1
Arthroplastic surgery	-	-	-	1
Weight gain and oedema	-	-	-	1
Total	44	25	12	39
Serious adverse events				
Necessitating discontinuation of study medication				
Gastrointestinal	1	1	NA	NA
Erythema annulare centrifugum	1	-	NA	NA
General unwellness	-	1	NA	NA
Dyspnea, insomnia, weight gain	-	1	NA	NA
Necessitating hospital admission				
Pancreatitis	1	-	-	-
Knee replacement surgery	1	-	-	-
Erosive arthritis	1	-	2	3
Meningitis	-	1	-	-
Venous thrombosis	-	-	1	-
Total	5	4	3	3

* Values are the number of events. DMARDs = disease-modifying antirheumatic drugs; RA = rheumatoid arthritis; MTX = methotrexate; NA = not available.

1 Discussion

2
3 This study indicates that MTX treatment of patients with UA can postpone progression
4 to RA, as defined by fulfillment of the ACR 1987 criteria, and can retard radiographic joint
5 damage. However, the results do not suggest that a 1-year course of MTX treatment can
6 prevent the development of RA from UA. Although these findings must be confirmed
7 in future trials, the PROMPT study provided the first evidence of the efficacy of MTX
8 treatment in UA patients.

9 Data from the current study showed that initiation of MTX treatment in UA patients
10 in the stage before they fulfilled the ACR criteria for RA resulted in postponement of the
11 diagnosis of RA. During the first year, the incidence of RA was lower in the MTX group
12 than in the placebo group, but after 12 months, the opposite was seen. Furthermore,
13 the results suggest that MTX did not induce more remission, but prolonged the period
14 of persistent UA. Interestingly, the benefit of this effect seemed to be the retardation
15 of radiographic progression. The majority (62%) of the 29 RA patients in the placebo
16 group had already fulfilled the ACR criteria and started taking open-label MTX within 6
17 months. Nevertheless, the results still show a more favorable outcome in the group that
18 immediately received MTX after study inclusion.

19 This is the first double-blind, randomized, placebo-controlled trial that addresses early
20 DMARD treatment in patients with UA before they fulfill the ACR criteria for established
21 RA. Although these criteria are currently under debate for use in clinical practice, for
22 research purposes they are regarded as the standard for objectively describing the RA
23 phenotype and have been widely used as inclusion criteria for trials. Moreover, results
24 from many randomized controlled trials have shown that in patients who fulfill the
25 ACR criteria for RA, DMARDs improve the outcome (18;19). Thus, for ethical reasons, a
26 placebo-controlled trial in UA patients could not be extended once the patients fulfilled
27 the criteria. Therefore, the primary outcome of the study, the diagnosis of RA as defined
28 by fulfillment of the ACR 1987 classification criteria, seems a reasonable end point.

29 Patients with UA included in this study are not completely representative of the av-
30 erage UA patient. This was illustrated by comparing the data from the patients in the
31 PROMPT study with controls from the Leiden EAC. Longer symptom duration and lower
32 ESR and CRP levels were seen in the patients in the PROMPT study. This indicates that
33 the UA patients were no longer in the earliest and most active disease state by the time
34 they were included in the current study. The best explanation for this observation seems
35 to be that physicians at the 4 centers were reluctant to expose patients with UA to a
36 1-year course of MTX treatment, given the high spontaneous remission rate and the
37 risk of unnecessary toxicity (3;20). Also, the use of the ACR 1958 criteria for RA as inclu-
38 sion criteria could have resulted in a selection of UA patients. Despite the fact that UA
39

1 patients already had longer disease duration, a 1-year course of MTX treatment was still
2 able to provide beneficial effects on disease and joint damage progression.

3 The existence of a therapeutic window of opportunity in UA patients, defined as a
4 period of time in which the disease process can be reversed, might not be demonstrated
5 in this study. However, it is possible that with a different design and a different medica-
6 tion scheme, such a therapeutic window can be addressed. First, the incidence of RA
7 increased during tapering of the study medication in the MTX group. This raises the
8 question of what would have happened if MTX had not been tapered. Second, MTX
9 as monotherapy could not have been sufficient, since in the MTX group, half of the RA
10 patients still developed RA while taking study medication, and 6 patients still showed
11 progression of joint damage. Trials in RA patients have shown that treatment with com-
12 bination therapy and/or biologic agents is more effective in preventing radiographic
13 joint damage (18;19;21). Finally, dosages of study medication were altered according to
14 the DAS because rheumatologists are generally satisfied and do not intensify therapy
15 when the DAS is <2.4 in RA patients (22;23). It is possible that treatment in UA patients
16 should aim at remission or a lower cutoff value of the DAS.

17 In this study, 53% of the patients in the placebo group developed RA and 24%
18 achieved spontaneous remission, demonstrating that MTX treatment is overtreat-
19 ment in a considerable proportion of UA patients. Because it is undesirable to start a
20 potentially harmful drug in UA patients who will remit spontaneously, there is a need
21 to identify those UA patients who will most likely develop RA and who will benefit the
22 most from DMARD treatment. In previous studies and in a recently published prediction
23 model that calculates the UA patients' risk of developing RA based on clinical variables,
24 the presence of anti-CCP emerged as one of the strongest predictors of RA (2;4;24).
25 Moreover, applying the model to our study, theoretically initiating treatment in patients
26 with a prediction score ≥ 8 and withholding treatment in patients with a prediction score
27 ≤ 6 , only 6% of the patients would have been inaccurately withheld from treatment, and
28 no patients would have been inaccurately treated.

29 In the current study, subgroup analysis revealed that the beneficial outcomes were
30 most pronounced in patients with anti-CCP. In striking contrast, in the anti-CCP-negative
31 subgroup, the effect of MTX on the development of RA, the radiographic progression,
32 and even on the signs and symptoms, was not demonstrable. The same observations
33 were made for patients who were or were not RF-positive, although this could reflect
34 the overlap with anti-CCP. Although the current groups are small, this post hoc analysis
35 suggests that only anti-CCP-positive UA patients, who have the highest risk of develop-
36 ing RA, benefit from early MTX treatment. It also supports the growing evidence that
37 anti-CCP-positive and anti-CCP-negative UA are different disease entities that should be
38 approached differently.

1 We conclude that treatment with MTX benefits patients with UA by reducing signs and
2 symptoms, by postponing the progression to RA as defined by the ACR 1987 criteria, and
3 by retarding radiographic joint damage. Furthermore, with the guidance of a prediction
4 model and the antibody status, it seems feasible to identify a subset of UA patients who
5 are most in need and who will benefit the most from initiation of MTX therapy, thereby
6 avoiding unnecessary toxic treatment. Although these findings have to be confirmed,
7 and the optimal duration and intensity of treatment still have to be determined, the
8 PROMPT study provides evidence for the efficacy of MTX treatment in UA patients.

10 **Acknowledgements**

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14 Netherlands Organisation for Scientific Research (NWO, grant 920-03-259 and 940-37-
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CHAPTER 5

Validity of the disease activity score in undifferentiated arthritis

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1 Summary

3 Objective

4 To study whether the disease activity score (DAS) is a valid measure of disease activity in
5 undifferentiated arthritis (UA).

7 Methods

8 Data from a double-blind placebo-controlled, randomized trial of MTX and placebo
9 involving 110 patients with UA were used. Data included baseline and 3, 6, 9, and 12
10 months, and diagnosis at 18 months. Validity of the DAS was analyzed using factor anal-
11 ysis, correlations with disease activity variables, correlations with changes in disability
12 and joint damage, differences in DAS between diagnoses, and detecting the difference
13 between placebo and MTX.

15 Results

16 Three disease activity factors were retrieved from the disease activity variables: patient
17 reported outcomes, tender and swollen joints, and acute phase reactants. The DAS
18 had its highest correlations ($r>0.77$) with tender joint counts, followed by swollen joint
19 counts ($r>0.63$) and patient reported outcomes ($r>0.30$), but correlated less with CRP
20 ($r=0.32$). DAS over time was related to HAQ response with an OR (95%CI) of 4.1 (2.1-8.0),
21 but not with change in joint damage. At 18 months, the mean DAS for RA patients was
22 2.6, for UA it was 2.2, and 1.9 for patients in remission ($p=0.001$). The DAS discriminated
23 better than all single variables between MTX and placebo, with a Guyatt's effect size of
24 0.89.

26 Conclusion

27 The DAS appears to be a reasonably valid measure of disease activity for use in UA clini-
28 cal trials.

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1 Introduction

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3 In Rheumatoid Arthritis (RA), the disease process develops before the diagnosis can be
4 made according to the current diagnostic criteria for RA (1,2). Indeed, depending on the
5 study population, 6-55% of patients with undifferentiated arthritis (UA) may develop
6 RA within 1 year (3). UA is defined as an inflammatory arthritis in which no definitive
7 diagnosis can (yet) be made. UA may be successfully treated before it develops into
8 erosive arthritis, such as RA (4). Until now, there is no valid measure of disease activity
9 in UA available. The Disease Activity Score (DAS) is a valid measure of RA disease activity
10 (5). The DAS may also be useful and valid in UA clinical trials, but the DAS has not been
11 formally tested in that setting.

12 The DAS was developed in RA patients, using data from the first 3 years after diagnosis
13 according to the ACR criteria (5). Although the variables included and the weights ap-
14 plied in the DAS were derived from early RA patients, later validation proved that the
15 DAS is also valid in patients with longer disease duration (6). UA covers a spectrum of
16 disease before the ACR classification criteria are fulfilled, and not all patients with UA
17 will eventually develop RA. Moreover, in UA generally less joints are involved, and the
18 pattern of involvement may differ from RA. Therefore, extended joint counts may be
19 more appropriate than reduced joint counts in UA.

20 The objective was to study whether the DAS is a valid measure of disease activity in
21 early undifferentiated arthritis, using the data from a randomised placebo-controlled
22 trial. The modified DAS (DAS28), which includes reduced joint counts, was also studied.
23 The alternative DAS28 modifications: Clinical Disease Activity Index (CDAI) or Simple
24 Disease Activity Index (SDAI) could not be calculated due to missing components.

26 Patients and methods

28 Trial data

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30 Use was made of data from the "PRObable rheumatoid arthritis: Methotrexate versus
31 Placebo Treatment" (PROMPT) trial that is described in more detail elsewhere (4). In sum-
32 mary, this study was a double-blind placebo-controlled, multi-centre, randomized trial
33 involving 110 patients with early undifferentiated arthritis (UA). Treatment started with
34 MTX (15 mg/week) or placebo tablets, and every 3 months the dose was increased if the
35 DAS was >2.4 . After 12 months, the study medication was tapered and discontinued in
36 patients not fulfilling the ACR criteria for RA. When a patient fulfilled the ACR criteria
37 for RA (primary endpoint), the study medication was changed to MTX. The diagnosis
38 at the end of the study and the progression of joint damage were prespecified primary
39 outcomes of this trial, to test whether MTX indeed modifies prognosis in UA (4).

1 Participants and assessments

2 Eligible patients attended the rheumatology out-clinic of the participating hospitals
3 and had symptoms of arthritis for not longer than 2 years, were at least 18 years of age,
4 and were diagnosed as having UA according to the American College of Rheumatology
5 (ACR) 1958 criteria for probable RA (7).

6 At baseline and 3, 6, 9, and 12 months, ESR and CRP, the 68 tender joint count, Ritchie Ar-
7 ticular Index, 66 swollen joint count, disability measurement (HAQ), and visual analogue
8 scales (0-100) for assessing pain, global disease activity, severity of morning stiffness,
9 fatigue, and general health were obtained. Every 6 months, radiographs of the hands and
10 feet were obtained, and scored according to the Sharp/van der Heijde scoring method.
11 At 18 months, the diagnosis was recorded by the patients' rheumatologist as RA, UA or
12 remission. Remission was defined as no clinical symptoms of arthritis and no DMARD use
13 the preceding year.

14 The disease activity score was calculated according to the original formula:

$$15 \text{ DAS} = 0.54 \times \sqrt{\text{RAI}} + 0.065 \times \text{SJC} + 0.33 \times \ln \text{ESR} + 0.0072 \times \text{GH},$$

16 where RAI is the Ritchie Articular Index, SJC is a 44 swollen joint count, ESR is the
17 Erythrocyte Sedimentation Rate, and GH is a patient assessed visual analogue scale
18 of General Health (0-100 mm.) (5). The RAI contains 53 joints and joint units that are
19 rated for tenderness on a scale from 0 (no tenderness), 1 (pain on pressure), 2 (pain
20 and winced), 3 (winced and withdrew). (8) For the 44 SJC, joints included in the RAI are
21 scored for swelling on a 0-1 scale, excluding TMJ joints, neck, hips, subtarsal and tarsal
22 joints. Levels of $\text{DAS} < 2.4$ are equated with "low" disease activity, and levels of $\text{DAS} > 3.7$
23 are equated with "high" disease activity (9). Similarly, the DAS28 was calculated using the
24 formula based on the 28-swollen and 28-tender joint count. (6).

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26 Statistical analysis

27 Cumulative frequency plots of the 68 tender joint count (TJC68), the 53 joint count of
28 the RAI without grading (TJC53), the 28 tender joint count (TJC28), and the 66, 44 and
29 28 swollen joint counts (SJC66, SJC44, SJC28) were produced to analyze the degree
30 of misclassification when using reduced joint counts. Differences between SJC66 and
31 SJC44, SJC44 and SJC28 at baseline and at 12 months were tested using the signed rank
32 test; similarly for TJC68 and TJC53, TJC53 and TJC28.

33 Analysis of the validity of the DAS was performed in 5 steps. For these analyses, data
34 were used from baseline and 3, 6, 9, and 12 months, and the final diagnosis obtained at
35 18 months. For comparison, the DAS28 was analyzed in the same way.

36 1) *Factor analysis*. The DAS had been devised to draw information from three underly-
37 ing factors of disease activity in RA: physical examination, laboratory values, and patient
38 reported outcomes (5). To reveal the factors underlying assessment of disease activity

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1 in UA, factor analysis with varimax rotation was used with a minimum eigen value >1 to
2 obtain factors using baseline data.

3 2) *Concurrent validity*. To inform how strong changes in the DAS within a patient are
4 correlated with changes in other variables supposed to assess disease activity, within-
5 subject correlations were calculated using linear regression with correction for repeated
6 observations, using the 5 visits of the first 12 months (10).

7 3) *Construct validity*. It was studied whether the DAS was related to changes in dis-
8 ability and to progression of joint damage over the first 12 months. Ordinal logistic re-
9 gression was used to relate the time-averaged DAS (0-12 months) to the HAQ response
10 at 12 months. The minimal clinical important difference of 0.22 in HAQ score was used
11 to classify patients as 'worsened', 'unchanged', or 'improved' (11). The proportional odds
12 assumption was checked by dichotomising the HAQ response into 'worsened' versus
13 'unchanged or improved', and 'worsened or unchanged' versus 'improved' and applying
14 two separate logistic regression models. It was checked graphically whether the logits
15 of the dichotomised HAQ responses were linearly related to the time-averaged DAS (12).
16 The Hosmer-Lemeshov test was used to indicate goodness of fit of both logistic regres-
17 sion models ($p > 0.05$), and a Chi-Square score test was used to test the proportional odds
18 assumption in the ordinal logistic regression model ($p > 0.05$) (12). Time-averaged DAS,
19 time-averaged CRP and the change in modified Sharp score over 0-12 months were ana-
20 lysed using logistic regression with progression > 3 points (yes/no) in modified Sharp
21 score as dependent variable and also using linear regression with change in modified
22 Sharp score as dependent variable.

23 4) *Criterion validity*. It was studied whether the mean DAS over the 5 visits in the first
24 12 months was different for patients who had erosive arthritis, undifferentiated arthritis,
25 or were in remission at visit 6 (18 months), using ANOVA and using ordinal logistic
26 regression. The underlying assumption is that disease activity, and mean DAS, is high-
27 est in patients developing erosive arthritis and lowest in patients going into remission.
28 The proportional odds assumptions for the ordinal logistic regression were tested in a
29 similar way as described above for HAQ responses.

30 5) *Responsiveness*. Responsiveness, or sensitivity to change, was calculated over the
31 visits at baseline and 12 months, using the Standardised Response Mean (SRM, mean
32 change / standard deviation of change), and Guyatt's responsiveness statistic (mean
33 change_{active treated group} / standard deviation_{placebo group}). Moreover, the t-value as a measure
34 of discrimination between the placebo and MTX trial arms was calculated.

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1 Results

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3 The values of the disease activity parameters at baseline of the study are shown in **Table**
4 **1**. The majority of the patients were female, the average age was about 50, 25% (27/110)
5 were positive for anti-CCP antibodies, and 39% (39/110) were rheumatoid Factor
6 positive. According to the distributions of the disease activity parameters in table 1, the
7 majority of patients were in moderate-to-low states of disease activity. There were 36%
8 (20/55) and 45% (25/55) patients with a DAS<2.4 at baseline. There were no statistically
9 significant differences between the trial arms at baseline in any of the parameters. The
10 DAS and the DAS28 had a Gaussian distribution (not shown).

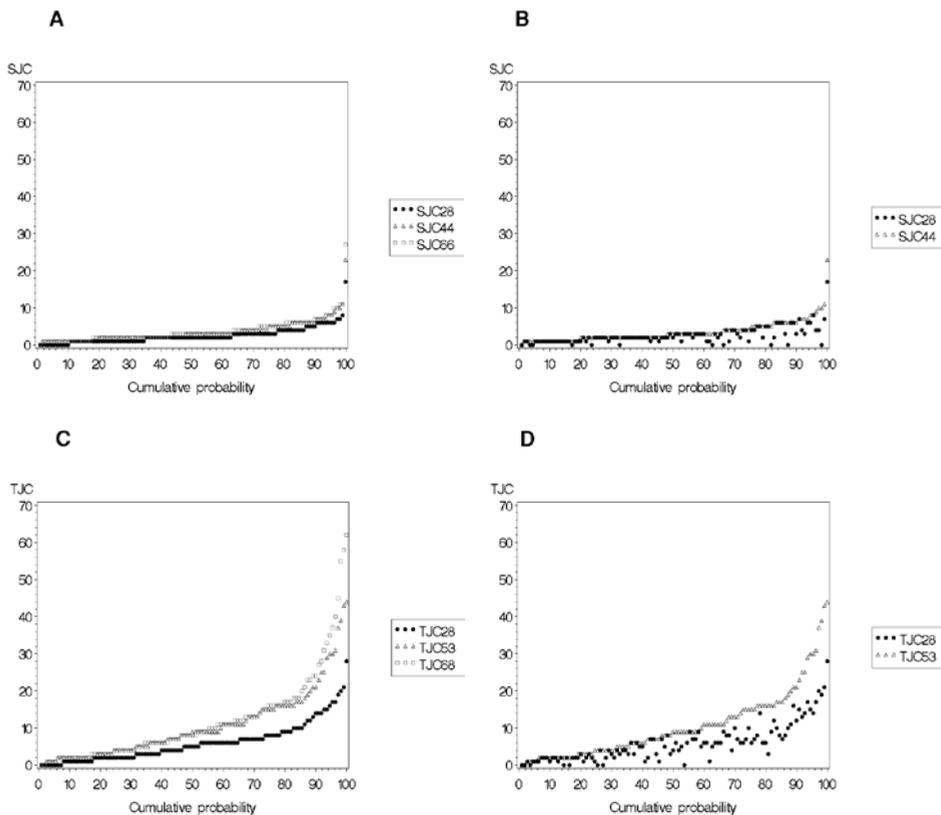
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14 **Table 1.** Disease activity parameters at baseline.

	MTX (n=55)		Placebo (n=55)	
15 Gender (female), n (%)	35	(64%)	38	(69%)
16 Age (year), mean (SD)	53	(14)	50	(14)
17 Symptom duration at first visit (days), median (IQR)	312	(195-507)	263	(169-432)
18 RF positive, n (%)	20	(36%)	19	(35%)
19 Anti-CCP positive, n (%)	12	(22%)	15	(27%)
20 ESR (mm/h), median (IQR)	12	(5-24)	11	(5-25)
21 CRP (mg/L), median (IQR)	5	(3-11)	5	(3-9)
22 SJC28, median (IQR)	2	(1-5)	2	(1-3)
23 SJC44, median (IQR)	3	(2-5)	2	(2-4)
24 SJC66, median (IQR)	3	(2-5)	3	(2-5)
25 TJC28, median (IQR)	6	(2-9)	4	(2-7)
26 TJC53, median (IQR)	11	(4-16)	7	(4-14)
27 RAI (0-78), median (IQR)	7	(3-10)	5	(2-8)
28 TJC68, median (IQR)	11	(4-17)	7	(4-14)
29 Pain (VAS), median (IQR)	44	(18-61)	48	(28-54)
30 Disease activity (VAS), median (IQR)	49	(15-60)	52	(29-64)
31 Morning stiffness severity (VAS), median (IQR)	45	(20-61)	47	(23-62)
32 Fatigue (VAS), median (IQR)	55	(22-76)	46	(9-72)
33 General health (VAS), median (IQR)	40	(19-52)	36	(16-55)
34 HAQ (0-3), median (IQR)	0.75	(0.38-1.13)	0.75	(0.25-1.13)
35 DAS, mean (SD)	2.72	(0.78)	2.52	(0.76)
36 DAS28, mean (SD)	3.94	(1.04)	3.72	(1.06)

37 Values are count (percentage) or median (interquartile range) or mean (SD). RF=rheumatoid factor,
38 Anti-CCP=anti-cyclic citrullinated peptide antibody, ESR=erythrocyte sedimentation rate, CRP=C-reactive
39 protein, SJC=swollen joint count, RAI=Ritchie articular index, TJC=tender joint count, VAS=visual analogue
scale, HAQ=disability index of the Health Assessment Questionnaire, DAS=disease activity score.

1 Cumulative frequency plots

2 In **figure 1**, a cumulative frequency plot of the 28, 44, and 66 swollen joint counts is
 3 shown at baseline (A). Every individual patient is represented in the curves as a single
 4 symbol, patients being ranked by the number of joints involved. The different kind of
 5 symbols denote the different joint counts, the patients do not necessarily have the same
 6 rank in the three curves. All differences between reduced and extended joint counts
 7 were statistically significant ($p \leq 0.002$), for the tender joint counts (B) as well as for the
 8 swollen joint counts (A) at baseline, and at 12 months (not shown).



32 **Figure 1.** Cumulative frequency plots of joint counts at baseline: average differences (A and B) and
 33 individual differences (C and D).

34 SJC=Swollen Joint Count; TJC=Tender Joint Count. Every individual patient is represented in the plots as a
 35 single symbol, patients being ranked by the number of joints involved. In panel A and B, the patients
 36 do not necessarily have the same rank in the three curves. The position of the median scores is where the
 37 cumulative frequency is 50%. In panel C and D, again every individual patient is represented in the plots
 38 as a single symbol, patients being ranked by their scores on the extended joint count. Only the median of
 39 the extended joint count is at a frequency of 50%.

The different joint counts are denoted by the different kind of symbols (see legend).

1 It can be seen in **figure 1 A** that the 66 SJC (squares) detects most swollen joints, with
 2 the 44 SJC (triangles) being close to it. The 28 SJC (filled dots) scores are somewhat lower
 3 than the other scores and the 28 SJC thus slightly underestimates the number of swollen
 4 joints. The number of patients with no swollen joints according to the 28 SJC and one
 5 or more swollen joints according to the extended joint counts does not exceed 10%
 6 at baseline, and at 12 months (not shown). **Figure 1 B** similarly shows the cumulative
 7 frequency plots of the 28, 53, and 68 tender joint counts at baseline. It can be seen
 8 that the number of tender joints involved is larger than the number of swollen joints.
 9 In figure 1 B, the scores of the tender joint counts follow the same trend, but the 28 TJC
 10 is the lowest. Misclassification (no tender joints according to the 28 TJC but at least 1
 11 tender joint according to the extended joint counts occurred in 6% at baseline (1 B) and
 12 11% at 12 months.

13 In **figure 1 C and D**, cumulative frequency plots are shown of the 28 and 44 SJC (C)
 14 and the 28 and 53 TJC (D). Now the patients are ordered by their scores on the extended
 15 joint count only. It can be seen in figure C that patients with no or few swollen joints
 16 according to the 28 SJC may have more than 1 swollen joint according to the 44 SJC. The
 17 same is seen for the 28 and 53 TJC in figure D.

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21 **Table 2.** Exploratory factor analysis at baseline.

	Factor		
	1	2	3
24 ESR (mm/h)	-0.05	0.02	0.88
25 CRP (mg/L)	-0.02	-0.09	0.88
26 SJC44	-0.15	0.60	-0.03
27 TJC53	0.18	0.93	-0.06
28 RAI (0-78)	0.28	0.85	-0.03
29 Pain (VAS)	0.86	0.06	0.10
30 Disease activity (VAS)	0.82	-0.08	0.08
31 Morning stiffness (VAS)	0.65	0.29	-0.14
32 Fatigue (VAS)	0.72	0.29	-0.12
33 General health (VAS)	0.85	-0.01	-0.13
34 HAQ (0-3)	0.53	0.57	0.06
35 Variance explained	46%	32%	22%

36 Three factors were derived using exploratory factor analysis with varimax rotation and a mineigen value
 37 >1 to obtain factors using baseline data. The factors may be named 1) 'patient reported outcomes', 2) 'joint
 38 counts', and 3) 'acute phase reactants'. For abbreviations see Table 1.

1 Factor analysis

2 By factor analysis, three factors were retrieved from the disease activity parameters at
3 baseline (**Table 2**). The first factor consisted of all the items on patient reported out-
4 comes, the second factor consisted of the examinations of tender and swollen joints, the
5 third factor included the acute phase reactants ESR and CRP. The HAQ loaded on both
6 the first and second factors. The ordering of factors in factor analysis and the amount of
7 variance explained also reflect the number of items included.

9 Concurrent validity

10 In **table 3**, the within-subject correlations of the DAS with other disease activity variables
11 are shown, using the visits of the first 12 months. The DAS had its highest correlations
12 with the RAI and the tender joint counts, it correlated well with the swollen joint counts
13 and most of the patient assessments and correlated less with CRP. Correlations of the
14 DAS28 generally were similar.

16 **Table 3.** Within-subject correlations with DAS and DAS28 over visits 1-5.

	DAS		DAS28	
	r	(95%CI)	r	(95%CI)
18 ESR (mm/h)	0.47	(0.31-0.61)	0.50	(0.34-0.63)
19 CRP (mg/L)	0.32	(0.14-0.48)	0.36	(0.19-0.52)
20 SJC28	0.62	(0.49-0.72)	0.63	(0.50-0.73)
21 SJC44	0.63	(0.50-0.73)	0.52	(0.37-0.65)
22 SJC66	0.62	(0.49-0.72)	0.50	(0.35-0.63)
23 TJC28	0.62	(0.49-0.72)	0.69	(0.58-0.78)
24 TJC53	0.71	(0.60-0.79)	0.63	(0.51-0.73)
25 RAI (0-78)	0.77	(0.68-0.84)	0.60	(0.47-0.71)
26 TJC68	0.64	(0.51-0.74)	0.57	(0.43-0.69)
27 Pain (VAS)	0.43	(0.26-0.57)	0.49	(0.33-0.62)
28 Disease activity (VAS)	0.43	(0.27-0.57)	0.48	(0.32-0.61)
29 Morning stiffness (VAS)	0.39	(0.22-0.54)	0.42	(0.25-0.56)
30 Fatigue (VAS)	0.30	(0.12-0.46)	0.35	(0.17-0.50)
31 General health (VAS)	0.46	(0.30-0.60)	0.54	(0.39-0.66)
32 HAQ (0-3)	0.35	(0.17-0.50)	0.35	(0.21-0.53)

33 The within-subject correlations inform about in how far a change in DAS (or DAS28) is paralleled by a
34 change in the variable in the left-hand column. For abbreviations see Table 1.

36 Construct validity

37 It was studied whether the time averaged DAS over 12 months was associated with
38 a change in HAQ over the same period of time, irrespective of treatment allocation
39 (**Figure 2**). HAQ scores were available of 102 patients. Over 12 months, there were 16

1 patients with a worsening in HAQ over 0.22 points, 40 patients with an improvement
 2 over 0.22, and 46 patients with smaller changes in either direction who were regarded
 3 as 'unchanged' (Figure 2). In ordinal logistic regression, the time averaged DAS was
 4 significantly associated with a minimal clinical important change (>0.22) in HAQ, with
 5 an OR (95%CI) of 4.1 (2.1-8.0), corrected for baseline HAQ, anti-CCP, age and gender
 6 as confounders. The assumptions for ordinal logistic regression, as outlined in the
 7 methods, were met. Further, it was studied whether time averaged disease activity and
 8 joint damage progression were correlated. However, there was no relation between
 9 time averaged CRP or time averaged DAS and change in Sharp score, irrespective of RF
 10 positivity, anti-CCP positivity, treatment, or final disease outcome (results not shown).

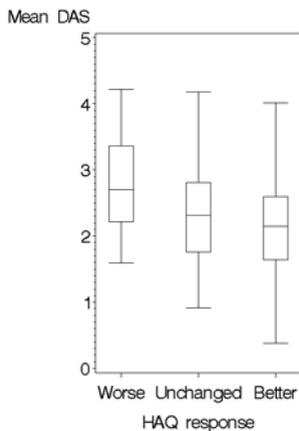


Figure 2. Response in HAQ and DAS scores averaged over time.

Box plots of the patient-averaged DAS over month 0-12, by HAQ response at month 12. HAQ response was defined as worsening (change in HAQ score > -0.22), no change (change in HAQ score between -0.22 and $+0.22$), and improvement (change in HAQ score $> +0.22$), according to (11).

Criterion validity

The level of disease activity is supposedly different for patients who develop RA, have undifferentiated arthritis, or reach remission. The mean (SD) DAS for patients with RA (n=49) was 2.6 (0.90), for patients with undifferentiated arthritis (n=21) it was 2.2 (0.72), for patients reaching remission (n=29) the DAS was 1.9 (0.64); $p=0.001$ for the differences between diagnostic groups. The OR (95%CI) for the differences between diagnostic groups in ordinal logistic regression was 2.5 (1.5-4.2) per time-averaged DAS point. A similar difference was observed for the DAS28. The assumptions for ordinal logistic regression were met for both analyses.

The mean (SD) DAS28 for patients with RA was 3.7 (1.09), for patients with undifferentiated arthritis it was 3.2 (1.91), for patients reaching remission the average DAS28 over time was 2.8 (0.79); $p=0.0007$ for the differences between diagnostic groups. At

1 18 months, 13 patients fulfilled the ARA criteria of remission. At that time, there were
 2 32 patients with a DAS<1.6, and 13/32 (40%) fulfilled the ARA criteria for remission. The
 3 number of patients with a DAS28<2.6 was 37, with 13/37 (35%) fulfilling the ARA criteria.

4 5 **Responsiveness**

6 The changes in disease activity parameters from baseline to 12 months are shown in
 7 Table 4. Disease activity improved in both treatment arms, but generally changes were
 8 larger in the MTX group. When adopting $p=0.05$ as critical value for determining sta-
 9 tistical significance, the critical value of t with this sample size is 2.0. In table 4 it can
 10 be seen that in the MTX group the DAS had a higher responsiveness (SRM) than the
 11 single parameters. The tender joint counts and the DAS and DAS28 showed the best
 12 performance in discriminating MTX from placebo according to Guyatt's responsiveness
 13 statistic and the t -value, with increasing discrimination with increasing number of joints
 14 assessed. The other variables did not exceed the critical t -value value of 2.0.

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16 **Table 4.** Responsiveness of disease activity parameters.

	MTX (n=55)			Placebo (n=55)			Guyatt's ES	t-value
	Mean	(SD)	SRM	Mean	(SD)	SRM		
19 DAS	-0.8	(0.8)	1.02	-0.4	(0.9)	0.40	0.89	2.40
20 DAS28	-1.1	(1.1)	1.05	-0.6	(1.3)	0.47	0.85	2.15
21 ESR (mm/h)	-5.6	(11.8)	0.47	-1.8	(15.2)	0.12	0.37	1.40
22 CRP (mg/L)	-3.6	(15.0)	0.24	-0.7	(17.0)	0.04	0.21	0.87
23 SJC28	-1.7	(2.6)	0.62	-1.2	(2.9)	0.42	0.57	0.77
24 SJC44	-1.9	(3.6)	0.53	-1.0	(4.7)	0.20	0.55	1.17
25 SJC66	-2.3	(4.5)	0.52	-1.4	(4.7)	0.29	0.55	1.08
26 TJC28	-2.7	(3.9)	0.68	-0.5	(5.2)	0.10	0.52	2.44
27 TJC53	-3.8	(6.4)	0.59	-0.31	(6.8)	0.04	0.57	2.71
28 RAI (0-78)	-3.3	(5.0)	0.65	-0.7	(4.4)	0.16	0.74	2.77
29 TJC68	-4.8	(7.8)	0.62	0.17	(9.0)	0.02	0.54	3.04
30 Pain (VAS)	-11	(25)	0.45	-15	(28)	0.52	0.41	0.59
31 Disease activity (VAS)	-10	(24)	0.41	-17	(28)	0.59	0.34	1.39
32 Morning stiffness (VAS)	-13	(25)	0.53	-15	(28)	0.54	0.46	0.49
33 Fatigue (VAS)	-7	(25)	0.27	-7	(30)	0.24	0.22	0.10
34 General health (VAS)	-9	(21)	0.44	-6	(31)	0.20	0.29	0.54
35 HAQ (0-3)	-0.19	(0.45)	0.42	-0.13	(0.47)	0.29	0.40	0.59

36 Shown are changes over 0-12 months in mean (SD). SRM=standardised response mean. For other
 37 abbreviations see Table 1.

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1 Discussion

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3 In this post-hoc analysis of the data from the PROMPT study of MTX versus placebo in
4 patients with early UA, it was studied whether the DAS is a valid measure of disease
5 activity in early UA. According to the results of this study, the DAS indeed appears to be
6 a reasonably valid measure of disease activity for use in UA clinical trials. However, the
7 validity of the DAS to measure arthritic disease activity in UA should also be tested in
8 other UA samples in different settings.

9 In absence of an unambiguous “gold standard” measure, validation took five steps:
10 factor analysis, concurrent validity, construct validity, criterion validity and respon-
11 siveness. The primary interest of this study in UA was in the DAS, because it includes
12 extended joint counts. The DAS28 with its reduced (28) joint counts was included to be
13 able to see whether or not there were large differences between the two measures in
14 their performance.

15 According to the comparison of reduced with extended joint counts in cumulative
16 frequency plots, it appeared that up to 10% of patients were misclassified as having no
17 swollen or tender joints by the 28 joint count. In contrast, these patients had 1 to 10
18 joints involved according to the extended joint counts. The largest difference between
19 28 and extended joint counts is that the former does not include the feet joints. Feet
20 joints may typically be involved in early RA (13). As the DAS includes extended joint
21 counts, the DAS may be preferred over the DAS28 in clinical trials in early RA or UA. It
22 could be shown in the frequency plots that the reduced joints are underestimating the
23 involvement of the number of joints. However, the lesser performance of the reduced
24 joint counts was not substantiated in the correlation of the DAS28 with other variables
25 and its responsiveness.

26 By the factor analysis, similar factors of disease activity were found in this study as
27 previously in RA (5). The three factors were patient reported outcomes, tender joints and
28 swollen joints, and acute phase reactants. Indeed, as can be seen in the DAS formula, the
29 DAS taps information from each of these constructs. Results of factor analysis depend
30 on the variables that are put in. The fact that a large proportion of the disease activity
31 parameters was patient-assessed, that naturally are closely correlated, will have contrib-
32 uted to retrieval of patient reported outcomes as the first factor.

33 In this study, the DAS correlated moderate-to-good with all other disease activity
34 parameters, including patient assessments, except for the acute phase reactants. The
35 contribution of the GH item to the DAS is relatively low (maximally 0.72 if GH is scored
36 100), which may be criticized as neglecting the patient perspective. However, despite
37 the weighting, the DAS is reasonably well associated with the other frequently used
38 patient assessments. The low within-subject correlations of the DAS with ESR and CRP
39 will be caused by the large proportion of patients who had acute phase reactants in

1 the normal range so changes in the acute phase reactants were not highly related to
2 changes in DAS or DAS28. On the other hand it is known that the DAS28 is sensitive for
3 changes in the low (even normal) range of ESR (14). The DAS over time was indeed re-
4 lated to a change in HAQ, as expected in advance. However, DAS over time and CRP over
5 time were both not associated with the progression of joint damage in this study. This
6 could not be explained, not even approximately, by differences in RF positivity, anti-CCP
7 positivity, treatment, or final disease outcome. However, the small number of patients
8 experiencing joint damage progression made it difficult to analyse these relations.

9 It was supposed in advance that the course of disease activity would be different
10 for patients who turned out to have RA, who still had UA, or who were in remission at
11 the end of the study. Indeed the mean DAS levels were clearly different for these three
12 diagnostic groups. The DAS and the DAS28 both did not perform well in predicting the
13 ARA criteria for clinical remission: all patients with ARA clinical remission had a DAS<1.6
14 and a DAS28<2.6, but only 40% of patients with a DAS<1.6 and 35% of patients with a
15 DAS28<2.6 fulfilled the ARA remission criteria.

16 The original PROMPT trial was powered for the primary outcome, which was the
17 diagnosis reached at 18 months (4). When analyzing the responsiveness of the several
18 disease activity parameters included as secondary outcomes, the DAS discriminated
19 well between MTX and placebo. Neither of the other parameters, except the tender joint
20 counts, reached statistical significance. Like in RA clinical trials, the pooling of informa-
21 tion in the DAS therefore may be advantageous for trials in UA.

22 An advantage of the DAS for assessment of disease activity is that it includes extended
23 joint counts. Whilst the 28 joint counts were introduced to facilitate a quick performance
24 of joint counts, their use may lead to an underestimation of disease activity if only few
25 joints are involved that are not among the 28 counted, such as the feet. This may play
26 a role in UA as well as in early RA. In UA, disease activity is generally low, and by using
27 extended joint counts including the feet an underestimation of the number of joints
28 involved may be prevented. For that reason, for clinical trials of early interventions in UA
29 or RA that aim at clinical remission the DAS may be preferred over the DAS28 (13). On
30 the other hand, in this validation study in UA, the DAS28 performed closely to the DAS
31 regarding all parameters of validity and responsiveness. It was the assessment of clinical
32 remission that was impeded by the use of reduced joint counts.

33 In summary, it can be concluded that the DAS, which is a valid measure of RA disease
34 activity, appears to be a reasonably valid measure of disease activity for use in UA clinical
35 trials. Similar factors of disease activity were found in this study as previously in RA; the
36 DAS correlated moderate-to-good with all other disease activity parameters including
37 patient-assessments; the DAS over time was related to changes in HAQ; the DAS was
38 different for patients with RA, UA and patients in remission; and the DAS discriminated
39 well between MTX and placebo. It may be regarded that the PROMPT trial we used was

1 relatively small (N=110) for validation purposes and therefore, studying the validity of
2 the DAS in UA in other samples is most welcome. The DAS may be used as primary or
3 secondary outcome measure for clinical trials in early UA. Instead of the DAS, the DAS28
4 may also be used, but the use of reduced joint counts underestimates the involvement
5 of inflamed joints.

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CHAPTER 6

A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis. How to guide individual treatment decisions

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1 **Abstract**

3 **Objective**

4 In patients with undifferentiated arthritis (UA), methotrexate is effective for inhibiting
5 symptoms, structural damage, and progression to rheumatoid arthritis (RA). How-
6 ever, 40-50% of patients with UA experience spontaneous remission. Thus, adequate
7 decision-making regarding treatment of patients with early UA requires identification of
8 those patients in whom RA will develop.

10 **Methods**

11 A prediction rule was developed using data from the Leiden Early Arthritis Clinic, an
12 inception cohort of patients with recent-onset arthritis (n = 1,700). The patients who
13 presented with UA were selected (n = 570), and progression to RA or another diagnosis
14 in this group was monitored for 1 year of followup. The clinical characteristics with
15 independent predictive value for the development of RA were selected using logistic
16 regression analysis. The diagnostic performance of the prediction rule was evaluated us-
17 ing the area under the curve (AUC). Cross-validation controlled for overfitting of the data
18 (internal validation). An independent cohort of patients with UA was used for external
19 validation.

21 **Results**

22 The prediction rule consisted of 9 clinical variables: sex, age, localization of symptoms,
23 morning stiffness, the tender joint count, the swollen joint count, the C-reactive protein
24 level, rheumatoid factor positivity, and the presence of anti-cyclic citrullinated peptide
25 antibodies. Each prediction score varied from 0 to 14 and corresponded to the percent
26 chance of RA developing. For several cutoff values, the positive and negative predictive
27 values were determined. The AUC values for the prediction rule, the prediction model
28 after cross-validation, and the external validation cohort were 0.89, 0.87, and 0.97,
29 respectively.

31 **Conclusion**

32 In patients who present with UA, the risk of developing RA can be predicted, thereby
33 allowing individualized decisions regarding the initiation of treatment with disease-
34 modifying antirheumatic drugs in such patients.

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1 Introduction

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3 Making individualized decisions regarding treatment is one of the most important chal-
4 lenges in medicine. To this end, several studies have associated clinical variables or gene
5 expression profiles with disease outcome, thereby providing help for clinicians making
6 treatment decisions in several diseases, e.g., breast cancer, Hodgkin's disease, and lym-
7 phoma (1-4). For the past decennium, treatment of rheumatoid arthritis (RA) has been
8 characterized by early, aggressive treatment with disease-modifying antirheumatic
9 drugs (DMARDs), because this treatment strategy prevents joint damage and functional
10 disability (5-7).

11 In rheumatology practices, the majority of patients who present with recent-onset
12 arthritis have undifferentiated arthritis (UA), which is a form of arthritis that does not
13 fulfill the classification criteria for a more definitive diagnosis. Based on results from sev-
14 eral inception cohort studies, it is known that ~40-50% of patients with UA experience
15 spontaneous remission, whereas RA develops in one-third of patients with UA (8-10).
16 Recent evidence indicates that treatment with methotrexate in patients with early UA
17 hampers progression to RA and progression of joint damage (11), underscoring the
18 need for guidance when starting treatment with a clinically beneficial but potentially
19 harmful drug in UA. Ideally, only the patients with UA in whom RA develops would be
20 treated with DMARDs, excluding those in whom UA remits spontaneously.

21 At present, although several risk factors for the development of RA have been identi-
22 fied (8;12), a model that predicts the disease course specifically in patients with recent-
23 onset UA is lacking. In the present study, we aimed to develop a model that predicts
24 progression from UA to RA, using clinical variables that are easily assessed in clinical
25 practice. The derived prediction rule was internally validated, controlling for overfitting
26 of the data, and was subsequently externally validated in an independent cohort of
27 patients with UA

28 29 30 Patients and methods

31 Patients

32 The prediction rule was derived using the Leiden Early Arthritis Clinic (EAC) cohort.
33 This inception cohort comprises more than 1,900 patients with recent-onset arthritis,
34 of whom ~1,700 have completed at least 1 year of followup. The EAC cohort began in
35 1993 at the Department of Rheumatology of the Leiden University Medical Center, the
36 only referral center for rheumatology in a health care region of ~400,000 inhabitants in
37 The Netherlands (13). General practitioners were encouraged to refer patients directly
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1 when arthritis was suspected; patients were included if a physical examination revealed
2 arthritis.

3 At the first visit, the rheumatologist completed a questionnaire regarding the present-
4 ing symptoms, as reported by the patient: type, localization and distribution of initial
5 joint symptoms, symptom duration, and course of the initial symptoms. The patient's
6 smoking history and family history were assessed. Patients rated morning stiffness on a
7 visual analog scale (VAS; range 0-100 mm). For the present study, the severity of morn-
8 ing stiffness was used instead of the duration of morning stiffness, because the former
9 variable has been proven to be a better discriminator (14;15). The Health Assessment
10 Questionnaire (16) was used to provide an index of disability. A 44-joint count for tender
11 and swollen joints was performed, scoring each joint on a 0-1-point scale (17). Compres-
12 sion pain in the metacarpophalangeal and metatarsophalangeal joints was recorded.

13 Baseline blood samples were obtained for determination of the erythrocyte sedimen-
14 tation rate (ESR), the C-reactive protein (CRP) level, the presence of IgM rheumatoid
15 factor (RF), as determined by enzyme-linked immunosorbent assay (ELISA), and the
16 presence of antibodies to cyclic citrullinated peptide 2 (anti-CCP), as determined by
17 ELISA (Immunoscan RA Mark 2; Euro-Diagnostica, Arnhem, The Netherlands). The cutoff
18 level for anti-CCP positivity was 25 arbitrary units. Radiographs of the hands and feet
19 were obtained and scored according to the Sharp/van der Heijde method (18). Patients
20 provided informed consent, and the local ethics committee approved the protocol.

21

22 **Assessment of disease status after 1 year**

23 Two weeks after being included in the study, when results of laboratory investigations
24 and radiography were known, 570 patients were determined to have a form of arthritis
25 that could not be classified according to American College of Rheumatology (ACR;
26 formerly, the American Rheumatism Association) criteria (19) and were documented
27 as having UA. After 1 year of followup, the disease status of all patients with UA was
28 examined to determine whether RA or another specific type of arthritis had developed,
29 based on fulfillment of the ACR criteria. Inherent in the design of an inception cohort,
30 the duration of followup differed within the study population, and at the moment of
31 analysis (July 2005), the majority of patients with UA (94%) had been followed up for
32 more than 1 year (mean \pm SD followup 8 ± 3 years).

33

34 **External validation cohort**

35 Patients included in the placebo arm of the Probaat (PROMPT) trial, a double-blind,
36 placebo-controlled, randomized trial in which patients with recent-onset UA were
37 treated with either methotrexate or placebo, were used for validation ($n = 55$) (11).
38 Exclusion of the patients with UA who were also included in the EAC cohort resulted in
39 36 independent patients with UA. Two of these patients were lost to followup. For each

1 patient, the progression score at baseline was calculated, and the development of RA
2 after 1 year of followup was assessed (11).

3

4 **Statistical analysis**

5 Patients with UA in whom RA developed were compared with those in whom RA did
6 not develop, using the chi-square test for nominal variables and Student's t-test for
7 continuous variables. Symptom duration was categorized. Subsequently, all clinical
8 variables were entered as possible explanatory variables in a logistic regression analysis,
9 with disease outcome (RA or non-RA) at 1 year of followup as the dependent variable.
10 Using a backward selection procedure, the most significant independent variables were
11 identified, using a *P* value greater than 0.10 as the removal criterion.

12 In the logistic regression model, the predicted probability of RA was related to the
13 covariates via the following prognostic index: $B_1 \times x_1 + B_2 \times x_2 + B_3 \times x_3 \dots B_k \times x_k$. The
14 regression coefficient (*B*) of the covariate indicates an estimate of the relative magnitude
15 of the prognostic power of a specific variable. Using the prognostic index, we calculated
16 the predicted probability of RA developing for every patient. For continuous variables
17 (age, VAS score, tender and swollen joint counts, CRP level), the effect was studied both
18 as a continuous variable and as a categorized variable. Categories were created using
19 clinically applicable cutoff levels and percentiles. Categories were pooled if correspond-
20 ing regression coefficients were similar. Data were missing for some patients, as follows:
21 morning stiffness score on a 100-mm VAS (*n* = 160 patients), anti-CCP antibody level
22 (*n* = 64 patients), disease duration (*n* = 22 patients), tender joint count (*n* = 5 patients),
23 swollen joint count (*n* = 4 patients), CRP level (*n* = 1 patient), and presence of RF (*n* = 1
24 patient). To prevent exclusion of these patients from the logistic regression analysis, the
25 median values for these variables were imputed.

26 To obtain a simplified prediction rule, the regression coefficients of the predictive
27 variables were rounded to the nearest number ending in .5 or .0, resulting in a weighted
28 score; subsequently, the values for the independent predictive variables were summed.
29 The calculated prediction scores were compared with the observed percentage of
30 patients who experienced progression to RA. The positive and negative predictive
31 values were determined for several cutoff values of the prediction scores. To evaluate
32 the diagnostic performance of the rule, a receiver operating characteristic (ROC) curve
33 was constructed. The area under the ROC curve (AUC) values provided a measure of the
34 overall discriminative ability of a model.

35 For internal validation, cross-validation was performed to control for overfitting (20).
36 Cross-validation mimics the prediction situation and for each observation yields a pre-
37 diction score based on the other (*n* - 1) observations (20). To validate the model, a ROC
38 curve was made using the cross-validated predictions as well as the external validation
39 cohort. SPSS version 10.0 software (SPSS, Chicago, IL) was used.

Table 1. Baseline characteristics of patients with UA, according to progression to RA*

Characteristic	No progression to RA (n = 393)	Progression to RA (n = 177)	P
Age, mean ± SD	48.6 ± 17.0	56.3 ± 15.3	<0.001
Female sex	208 (53)	121 (68)	0.001
Positive family history of RA	81 (21)	54 (31)	0.01
Course of starting symptoms			
acute for <24 hours	116 (30)	36 (20)	
subacute for > 24 hours	123 (31)	51 (29)	
gradual	141 (36)	86 (49)	
intermittent	13 (3)	4 (2)	0.02
Symptom duration at inclusion			
< 6 weeks	103 (27)	18 (11)	
6 weeks to 3 months	80 (21)	43 (25)	
3 to 6 months	89 (23)	47 (28)	
> 6 months	107 (28)	61 (36)	<0.001
Localization of affected joints			
small joints	171 (44)	95 (54)	
large joints	165 (42)	32 (18)	
small and large joints	57 (15)	50 (28)	<0.001
Localization of affected joints symmetric	147 (37)	118 (67)	<0.001
Localization of affected joints			
upper extremities	177 (45)	71 (40)	
lower extremities	139 (35)	22 (12)	
upper and lower extremities	77 (20)	84 (47)	<0.001
Morning stiffness, mean ± SD score on a 100-mm VAS	35.5 ± 30.0	53.3 ± 30.1	<0.001
Compression pain in MCP joints	159 (40)	116 (66)	<0.001
Compression pain in MTP joints	134 (34)	103 (58)	<0.001
Number of tender joints, median (IQR)	5 (2-11)	11 (7-22)	<0.001
Number of swollen joints, median (IQR)	2 (1-4)	4 (2-7)	<0.001
C-reactive protein level, median (IQR) mg/liter	8 (3-21)	14 (7-43)	<0.001
ESR, median (IQR) mm/hour	17 (8-38)	32 (19-53)	<0.001
Rheumatoid factor positivity	56 (14)	84 (47)	<0.001
Anti-CCP positivity	38 (11)	83 (51)	<0.001
HAQ score, mean ± SD	0.7 ± 0.6	1.0 ± 0.7	<0.001
Smoking	187 (48)	84 (47)	1.0
Erosive disease	29 (7)	29 (16)	0.001

* Except where indicated otherwise, values are the number (%). UA = undifferentiated arthritis; RA = rheumatoid arthritis; VAS = visual analog scale; MCP = metacarpophalangeal; MTP = metatarsophalangeal; IQR = interquartile range; ESR = erythrocyte sedimentation rate; anti-CCP = anti-cyclic citrullinated peptide; HAQ = Health Assessment Questionnaire.

1 Results

3 Disease outcome

4 During the first year of followup, RA developed in 177 of the 570 patients with UA, other
 5 rheumatologic disease developed in 94 patients, and 150 patients achieved clinical
 6 remission, defined as discharge from the outpatient clinic because of the absence of ar-
 7 thritis while not receiving DMARD treatment. For further analysis, patients with another
 8 rheumatologic diagnosis or UA and those who achieved remission were assembled as
 9 the non-RA group (n = 393).

11 **Table 2.** Independent predictive variables for development of RA based on results of multivariate
 12 regression analysis*

13	Variable	B	OR	95%CI	P	Points†
14	Sex	0.8	2.1	1.3-3.6	0.003	1
15	Age	0.02	1.02	1.01-1.04	0.011	0.02/year
16	Localization small joints hand/feet	0.6	1.8	1.1-3.1	0.024	0.5
17	Symmetric localization	0.5	1.6	1.0-2.8	0.075	0.5
18	Localization in upper extremities	0.8	2.1	1.1-4.4	0.04	1
19	Localization in both upper and lower extremities	1.3	3.5	1.7-7.5	0.001	1.5
20	Morning stiffness score on 100-mm VAS					
	0-25	-	-	-	-	-
21	26-50	0.9	2.4	1.2-4.5	0.009	1
22	51-90	1.0	2.7	1.3-5.6	0.006	1
	>90	2.2	9.3	3.0-28.7	<0.001	2
23	Number of tender joints					
24	0-3	-	-	-	-	-
25	4-10	0.6	1.8	0.9-3.3	0.082	0.5
26	>10	1.2	3.3	1.5-7.0	0.003	1
27	Number of swollen joints					
	0-3	-	-	-	-	-
28	4-10	0.4	1.5	0.8-2.7	0.18	0.5
29	>10	1.0	2.8	1.1-7.6	0.038	1
30	CRP level, mg/liter					
	0-4	-	-	-	-	-
31	5-50	0.6	1.6	0.9-3.0	0.13	0.5
32	>50	1.6	5.0	2.0-12.1	0.00	1.5
33	RF positivity	0.8	2.3	1.2-4.2	0.009	1
34	Anti-CCP positivity	2.1	8.1	4.2-15.8	<0.001	2

35 * B values are regression coefficients. RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence
 36 interval; VAS = visual analog scale; CRP = C-reactive protein; RF = rheumatoid factor; anti-CCP = anti-cyclic
 37 citrullinated peptide.

38 † For the simplified prediction rule derived from the regression coefficient.

1 Univariate analyses

2 The characteristics of patients with UA in whom RA developed and those in whom RA
3 did not develop are compared in **Table 1**. In the univariate analyses, all variables except
4 smoking were significantly associated with progression to RA.

6 Multivariate analyses and derivation of the prediction rule

7 In the logistic regression analysis, the independent predictive variables for development
8 of RA were age, sex, localization of joint symptoms (small/large joints, symmetric/asym-
9 metric, upper/lower extremities), morning stiffness, tender and swollen joint counts,
10 CRP level, and the presence of RF or anti-CCP antibodies (Table 2).

11 Because age was more predictive as a continuous variable than as a categorized vari-
12 able, age was not categorized. The other variables were categorized. For the resulting
13 model, the fraction of explained variation (Nagelkerke's R²) was 0.57; when using a
14 predicted probability of 0.5 as the cutoff value, the outcomes for 83% of patients were
15 predicted correctly. The coefficients for the simplified prediction score are listed in **Table**
16 **2**. **Figure 1** presents a form that can be used to easily calculate the prediction score. The

17			
18			
19	1. What is the age in years?	Multiply with 0.02	_____
20	2. What is the sex?		
21		In case female:	1 point _____
22	3. How is the distribution of involved joints?		
23		In case small joints hands/feet:	0.5 point _____
24		In case symmetric:	0.5 point _____
25		In case upper extremities	1 point _____
26		In case upper and lower extremities:	1.5 points _____
27	4. What is the score for morning stiffness on a 100-mm VAS?		
28		In case 26-90 mm:	1 point _____
29		In case >90 mm:	2 points _____
30	5. What is the number of tender joints?		
31		In case 4-10:	0.5 point _____
32		In case 11 or higher:	1 point _____
33	6. What is the number of swollen joints?		
34		In case 4-10:	0.5 point _____
35		In case 11 or more:	1 point _____
36	7. What is the C-reactive protein level?		
37		In case 5-50 mg/liter:	0.5 point _____
38		In case 51 mg/liter or higher:	1.5 points _____
39	8. Is the patient rheumatoid factor positive?		
		If yes:	1 point _____
	9. Are the anti-CCP antibodies positive?		
		If yes:	2 points _____
		Total score	_____

Figure 1. Form used to calculate a patient's prediction score. The range of possible scores is 0-14, with higher scores indicating a greater risk of developing rheumatoid arthritis. VAS = visual analog scale; anti-CCP = anti-cyclic citrullinated peptide.

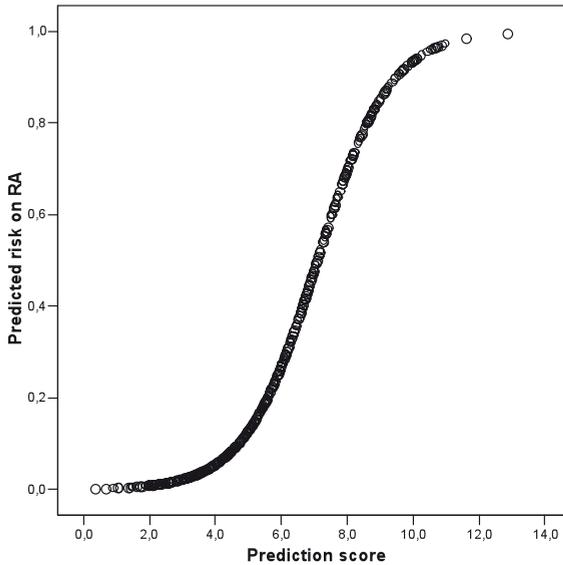


Figure 2. Predicted risk of rheumatoid arthritis (RA) as a function of the prediction score.

range of the prediction scores is 0-14, with a higher score indicating a greater risk of developing RA.

A prediction score was calculated for every patient with UA. **Figure 2** shows the predicted risk of RA as a function of the prediction score (obtained from a logistic regression model with score as the independent variable). **Table 3** shows the observed percentage of patients who experienced progression to RA in relation to the calculated prediction score. None of the patients with UA who had a prediction score ≤ 3 progressed to RA during the 1-year followup period, and all of the patients with UA who had a score ≥ 11 did experience progression to RA. Among the patients with scores of 4-10 who experienced progression to RA, the frequency of such progression increased with rising scores.

Table 4 shows the percentage of patients in whom RA developed, according to several cutoff values of the prediction score. For example, when the scores 5.0 and 9.0 were chosen as cutoff values, 97% of patients with UA who had a score ≤ 5.0 did not develop RA, and a score ≥ 9.0 was associated with progression to RA in 84% of patients. When cutoff values of 6.0 and 8.0 were used, 91% of patients with UA who had a score ≤ 6.0 did not develop RA (negative predictive value 91%, 95% confidence interval [95% CI] 88-94%), and a score ≥ 8.0 corresponded to progression to RA in 84% of patients (positive predictive value 84%, 95% CI 75-91%). Using these cutoff values, 145 patients with UA (25%) had a score between 6.0 and 8.0, indicating that no adequate prediction could be made for these patients. Twenty-five patients with UA did not fulfill the 1987 ACR criteria for RA after 1 year of followup, but RA developed later in the disease course. These patients had a median prediction score of 5.7 (interquartile range [IQR] 4.8-6.2); this value is between

Table 3. Prediction scores and progression or nonprogression to RA*

Prediction score	No progression to RA (n = 387)	Progression to RA (n = 175)
0	1 (100)	0 (0)
1	8 (100)	0 (0)
2	42 (100)	0 (0)
3	58 (100)	0 (0)
4	78 (93)	6 (7)
5	73 (85)	13 (15)
6	63 (74)	22 (26)
7	37 (49)	38 (51)
8	16 (33)	33 (67)
9	6 (14)	36 (86)
10	5 (23)	17 (77)
11	0 (0)	8 (100)
12	0 (0)	1 (100)
13	0 (0)	1 (100)
14	0 (0)	0 (0)

* Values are the number (%) of patients with a given score. Scores were rounded to the nearest number ending in .5 or .0 (i.e., scores ≤ 0.5 are in the category 0, scores >0.5 and ≤ 1.5 are in the category 1, etc.). RA = rheumatoid arthritis.

Table 4. Cutoff values for prediction scores and risk of development of RA*

Cutoff values	No progression to RA	Progression to RA
Score ≤ 4.0	145 (99)	1 (1)
4.0 -10.0	240 (60)	159 (40)
≥ 10.0	2 (12)	15 (88)
Score ≤ 5.0	223 (97)	8 (3)
5.0-9.0	157 (55)	131 (46)
≥ 9.0	7 (16)	36 (84)
Score ≤ 6.0	296 (91)	28 (9)
6.0-8.0	76 (52)	69 (48)
≥ 8.0	15 (16)	78 (84)

* Values are the number (%) of patients with a given score. Scores were rounded to the nearest number ending in .5 or .0. RA = rheumatoid arthritis.

the scores for the patients with UA in whom RA developed and those in whom RA did not develop during the first year of followup (median score 7.7 [IQR 6.6-8.8] and median score 4.6 [IQR 3.3-5.9], respectively).

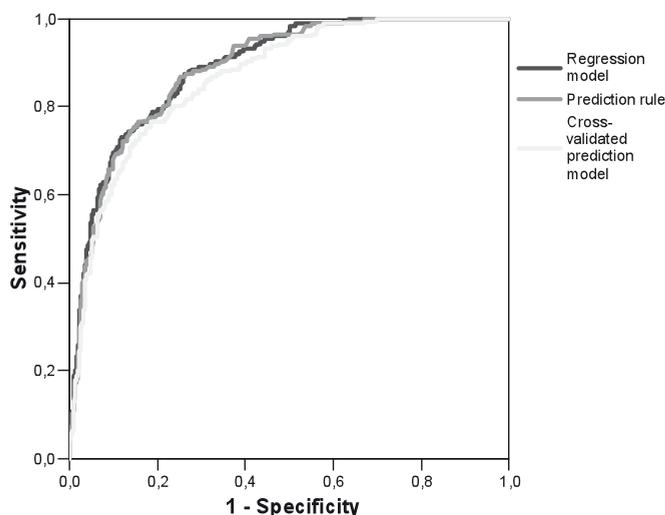
Discriminative ability

The discriminative ability of the logistic regression model and the prediction rule were evaluated with a ROC curve (Figure 3). Both the logistic regression model and the pre-

1 diction rule had a mean \pm SD AUC value of 0.89 ± 0.014 . The finding that the AUC values
 2 for the logistic regression model and the prediction rule were equal indicates that the
 3 derivation of the prediction rule from the logistic regression model had not introduced
 4 a loss of discriminative ability.

5 6 Internal validation

7 Cross-validation was used to control for overfitting. This procedure yielded a value for
 8 the predicted probability of RA for every patient, based on results of model-fitting on
 9 the other patients (20). The AUC value of the cross-validated predictions nearly equaled
 10 the mean \pm SD AUC value of the prediction score (0.87 ± 0.015) (**Figure 3**), indicating that
 11 overfitting was not a major problem.



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Figure 3. Receiver operating characteristic curve for the logistic regression model, the prediction rule, and the cross-validated prediction model. The area under the curve values for the logistic regression model, the prediction rule, and the cross-validated prediction model were 0.89, 0.89, and 0.87, respectively.

30 External validation

31 In the validation cohort, 47% of patients with UA had experienced progression to RA
 32 after 1 year of followup. The prediction scores for the patients with UA in whom RA did
 33 develop and those in whom RA did not develop are presented in **Figure 4**. Among patients
 34 with UA who experienced progression to RA, the median prediction score was 8.0
 35 (IQR 6.1-9.1); among patients in whom RA did not develop, the median prediction score
 36 was 4.6 (IQR 3.5-5.5). Ninety-four percent of the patients with a prediction score ≤ 6.0
 37 had not experienced progression to RA, and the development of RA was observed in
 38 83% of patients with a score >6 . All patients with a score ≥ 8.0 had progressed to RA, and
 39 78% of patients with a score <8 did not develop RA. In the validation cohort, 17% of the

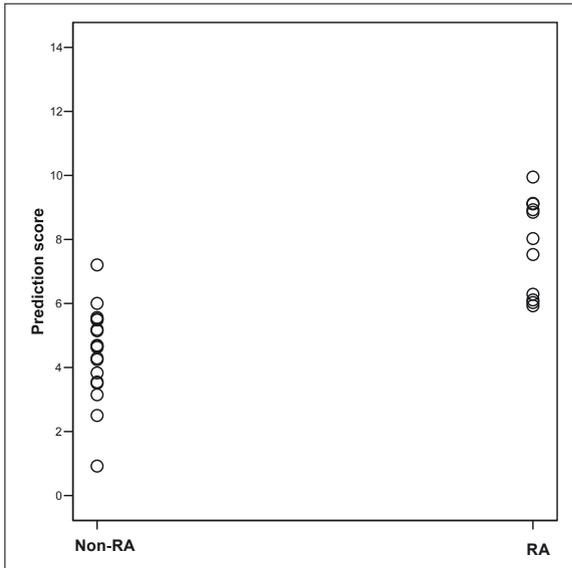


Figure 4. Prediction scores for patients with undifferentiated arthritis in whom rheumatoid arthritis (RA) did develop and those in whom RA did not develop.

patients with UA had a prediction score between 6 and 8; RA had not developed in two-thirds of these patients and had developed in one-third of them. If treatment decisions were based on the prediction rule using cutoff levels of ≥ 8 for initiating treatment and ≤ 6 for withholding treatment, treatment would have been withheld inaccurately from only 6% of the patients, and no patient would have received treatment inaccurately. The mean \pm SD AUC value for the validation cohort was 0.97 ± 0.024 .

Discussion

The currently developed rule predicts the development of RA in patients with UA, using 9 clinical variables that are commonly assessed during the first visit: sex, age, localization of joint symptoms, morning stiffness, tender and swollen joint counts, the CRP level, and the presence of RF and anti-CCP antibodies. The resulting prediction score corresponds to a chance for progression to RA. The positive and negative predictive values of the prediction score depend on the chosen cutoff values. The discriminative ability was excellent, with an AUC value of 0.89 and with a value of 0.87 after internal validation correcting for overfitting. Subsequent validation in a small independent cohort revealed an AUC value of 0.97. Because the prediction rule is accurate and can be easily determined in daily clinical practice, the present model is an important step forward in achieving individualized treatment in patients with recent-onset UA.

1 Because current evidence regarding treatment of RA is based on results of large trials
2 involving patients fulfilling the ACR 1987 revised criteria for RA (19), fulfillment of these
3 criteria was used as the outcome for the current study. Alternative outcome measure-
4 ments such as disease persistence or remission can be considered, but no generally
5 accepted definitions of these disease states are available, and no trials of patients with
6 these disease states are available to provide guidance when making treatment deci-
7 sions. Nevertheless, the use of fulfillment of the ACR criteria as outcome may lead to
8 circularity, because use of the items included in the ACR criteria is expected to result in
9 the identification of predictive variables. However, several studies have shown that the
10 ACR criteria themselves have low discriminative value in patients with UA (12;21-25),
11 and only some of the variables used for the present prediction rule are among the ACR
12 criteria. In the end, it will most likely not make a large difference whether the outcome of
13 a prediction rule is the diagnosis RA or disease persistence, because the ACR criteria are
14 formulated based on patients with longstanding/persistent RA (mean disease duration
15 8 years), and the reported remission rate in these patients is low (10-15%) (26;27).

16 Misclassification may have occurred when patients who presented with UA were
17 treated with any drug that hampered the progression to RA. In such cases, patients who
18 normally would have progressed to RA would be classified as non-RA. Exclusion of such
19 misclassified patients, who supposedly would have high prediction scores because they
20 were prone to the development of RA, will result in an increased discriminative ability of
21 the current prediction rule.

22 The presence of erosions on radiographs of the hands and/or feet is reported to
23 have high specificity (but low sensitivity) for discriminating between self-limiting and
24 persistent disease (25). Although in univariate analysis, the presence of erosions was
25 significantly increased in UA patients in whom RA developed compared with patients
26 in whom RA did not develop (16% versus 7%), multivariate regression analysis revealed
27 that the presence of erosions was not an independent prognostic variable. The presence
28 of erosions appeared to be associated with a higher median age (64 years in patients
29 with erosive disease versus 49 years in those with nonerosive disease), a higher median
30 number of swollen joints (5 joints in patients with erosive disease versus 2 joints in those
31 with nonerosive disease), and the presence of RF (46% of patients with erosive disease
32 versus 23% of those with nonerosive disease). Because the presence of erosions was not
33 identified as a variable with an independent predictive value, data on erosions were not
34 included in the prediction rule.

35 A model for predicting self-limiting, persisting, or erosive arthritis exists (25). For the
36 development of that model, all consecutive patients with arthritis who were referred
37 were incorporated, including patients in whom a definite diagnosis was made during
38 the first weeks. Decisions regarding the initiation of DMARDs are seldom problematic in
39 such patients. At present, support is needed in making treatment decisions for patients

1 with recent-onset UA (28), because the disease outcome in patients with UA is variable.
2 For the present study, we selected patients with UA from a total of 1,700 consecutive
3 patients and developed a prediction rule specifically for UA.

4 The positive and negative predictive values of the prediction score depend on the
5 chosen cutoff level. When the upper and lower cutoff values were 8.0 and 6.0, the cor-
6 responding positive predictive value and negative predictive value were 84% and 91%,
7 respectively. In the original cohort, 25% of patients had a prediction score between
8 6.0 and 8.0; in these patients, the chance of RA developing or not developing was
9 equal. Apparently, the clinical characteristics of patients with intermediate scores are
10 insufficient to predict disease outcome. It is possible that genotype data are helpful in
11 these patients. Patients were also typed for HLA-DRB1 shared epitope (SE) alleles and
12 PTPN22. In the multivariate analysis, the presence of SE alleles was not identified as
13 an independent predictive variable; this might be attributable to the fact that the SE
14 alleles are associated with the presence of anti-CCP antibodies (29), which are already
15 included in the prediction rule. Also, the presence of the PTPN22 T allele did not result
16 in a better ability to predict progression from UA to RA. This is understandable, because
17 the PTPN22 T allele confers risk of both UA and RA (30). In the validation cohort, 17% of
18 patients had a prediction score between 6.0 and 8.0; for treatment decisions in these
19 patients the observed risk of progression to RA can be weighted against the individual
20 risk profile for treatment toxicity.

21 The prediction score discriminated even better in the validation cohort than in the ini-
22 tial cohort: 100% of patients with a score ≥ 8.0 had progressed to RA, and 94% of patients
23 with a score ≤ 6.0 did not develop RA. This indicates that if treatment decisions were
24 based on the prediction rule using the cutoff levels of ≥ 8 for initiating treatment and
25 ≤ 6 for withholding treatment, treatment would be inaccurately withheld in only 6% of
26 patients, and no patient would receive treatment inaccurately. Although the validation
27 cohort is relatively small and the current prediction rule should be evaluated in other
28 early arthritis cohorts, we believe that the current model allows physicians and patients
29 to make an evidence-based choice regarding whether or not to initiate DMARDs in the
30 majority of patients presenting with UA.

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CHAPTER 7

Pretreatment serum levels of anti-cyclic citrullinated peptide antibodies are associated with the response to methotrexate in recent-onset arthritis

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To direct individual treatment decisions in recent-onset rheumatoid arthritis (RA), predictors of treatment response to methotrexate (MTX) need to be identified. Disease activity at baseline, gender and genetic polymorphisms have already been found to be associated with the effect of MTX treatment, but the predictive value of autoimmune antibody status remains less clear(1;2). It has been shown, however, that both the presence and level of anti-cyclic citrullinated peptide antibodies (ACPA) are strongly associated with a worse disease course (3). Therefore, we investigated the potential predictive effect of levels of ACPA in ACPA-positive patients for the response to MTX treatment. As observations from our cohort and others indicate that ACPA levels decrease during treatment, we studied two selected populations of disease-modifying antirheumatic drug (DMARD)-naïve, ACPA-positive patients with recent-onset arthritis, for whom pretreatment ACPA levels were available (4;5).

All ACPA-positive patients with undifferentiated arthritis (UA) who were included in the PROMPT study (PRObable RA: Methotrexate versus Placebo Treatment) (MTX group n=12, placebo group n=15) were enrolled (5). MTX treatment was started with 15 mg/week and every 3 months the dosage was increased according to the Disease Activity Score (DAS44) to a maximum of 30 mg/week. Responders were defined as patients whose UA did not progress to RA (according to the American College of Rheumatology criteria) during the use of MTX (n=6/12). MTX responders had lower levels of pretreatment IgG ACPA than non-responders (median (interquartile range) 428 (214–643) AU/

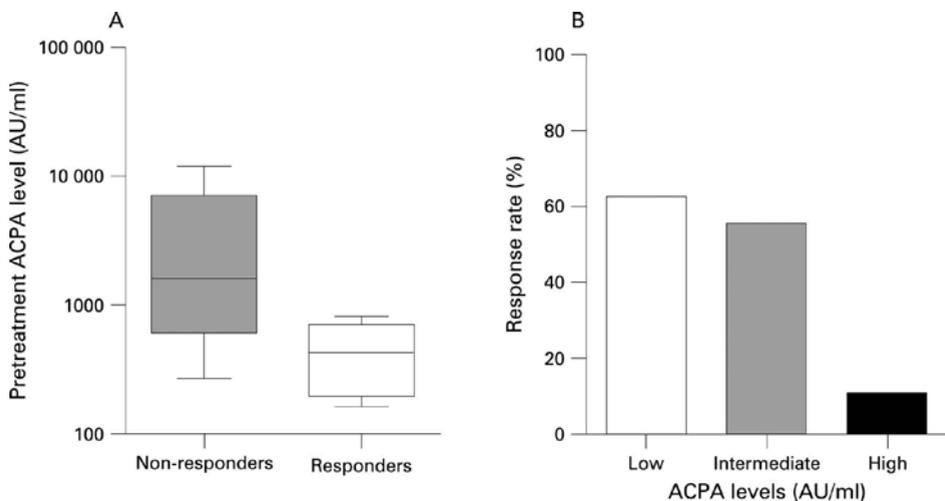
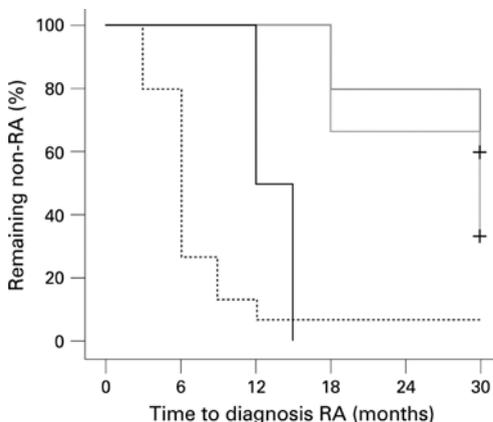


Figure 1. (A) Pretreatment anti-cyclic citrullinated peptide antibodies (ACPA) levels in methotrexate (MTX) responders versus non-responders within ACPA-positive patients with recent-onset, undifferentiated arthritis from the PROMPT study (n=12, p=0.024). (B) Percentage of responders to MTX in ACPA-positive patients with recent-onset rheumatoid arthritis from the BeSt study with low, intermediate and high pretreatment ACPA levels (n=26, p=0.062).

1 ml vs 1594 (781–4495) AU/ml, respectively) ($p=0.024$, Mann–Whitney test, **figure 1A**.
 2 Further univariate analysis did not show any significant differences in baseline clinical
 3 measures of disease activity, or in rheumatoid factor levels, between responders and
 4 non-responders. In addition, the risk of progression to RA, as analysed by survival analy-
 5 sis, was lower in patients with low or intermediate pretreatment ACPA levels than in
 6 patients with high levels (stratified by tertiles) ($p<0.001$, log-rank test, **figure 2**).

7 Similar associations were found in a second cohort of ACPA-positive patients with
 8 recent-onset RA, who were treated with initial MTX monotherapy (15–25 mg) aiming at
 9 a DAS44 ≤ 2.4 in the BeSt study and from whom pretreatment serum samples were avail-
 10 able ($n=26/131$) (6). Responders were defined as patients achieving a DAS44 ≤ 2.4 after
 11 6 months. The percentage of responders decreased from 63% and 56% in patients with
 12 low and intermediate levels, respectively, to 11% in patients with high levels (stratified
 13 by tertiles) ($p=0.062$, χ^2 test, **figure 1B**). In a multivariate logistic regression analysis, low
 14 and intermediate ACPA levels predicted responsiveness, independently of baseline DAS,
 15 gender and age (odds ratio=37, 95% confidence interval 0.8 to 1692, $p=0.064$).

16 Despite the limited number of patients, these data from two distinct cohorts suggest
 17 that low and intermediate pretreatment levels of ACPA are associated with a more
 18 favourable response to MTX treatment in recent-onset, ACPA-positive arthritis, whereas
 19 high levels are associated with an insufficient response. Although these findings have to
 20 be confirmed in larger studies, quantitative evaluation of ACPA levels might be an addi-
 21 tional tool to determine which patients will benefit most from MTX treatment. Therefore,
 22 we propose that pretreatment ACPA levels should be used in future prediction analyses.



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 36 **Figure 2.** Kaplan–Meier survival curves for the progression of undifferentiated arthritis to rheumatoid
 37 arthritis (RA) in anti-cyclic citrullinated peptide antibody (ACPA)-positive patients with undifferentiated
 38 arthritis. Placebo group (broken line, $n=15$); methotrexate group (solid lines, $n=12$; categorised into
 39 patients with low (light line), intermediate (semi-dark line) and high (dark line) pretreatment ACPA levels
 by tertiles). $p<0.001$ for the comparison of low/intermediate versus high levels.

1 Acknowledgements

2
3 The PROMPT study was financed with grants from the Dutch Arthritis Foundation and
4 the Netherlands Organisation for Scientific Research. The BeSt study was financed with
5 grants from the Dutch College of Health Insurances, Schering-Plough BV and Centocor
6 Inc.

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CHAPTER 8

Suppressor activity among CD4+, CD25++ T cells is discriminated by membrane- bound tumor necrosis factor alpha

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1 Abstract

2

3 Objective

4 Previous studies have shown that the suppressive capacity of CD4+,CD25++ T cells is
5 compromised in patients with rheumatoid arthritis (RA) and restored by anti-tumor
6 necrosis factor α (anti-TNF α) therapy. Given the lack of specific cell surface markers for
7 human Treg cells, this study aimed to define surface markers for identifying and enrich-
8 ing Treg cells with enhanced regulatory ability within the CD4+,CD25++ T cell compart-
9 ment and to provide additional understanding of the effects of anti-TNF α antibodies in
10 humans.

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12 Methods

13 The expression of membrane-bound TNF α in human peripheral blood CD4+ T cells was
14 analyzed by flow cytometry in healthy individuals and RA patients before and after anti-
15 TNF α treatment. Membrane-bound TNF α -positive and TNF α -negative CD4+,CD25++ T
16 cells were purified by fluorescence-activated cell sorting, and their suppressive capacity
17 was assessed in vitro by a standard suppression assay.

18

19 Results

20 A substantial number of CD4+,CD25++ T cells expressed membrane-bound TNF α .
21 Membrane-bound TNF α -positive CD4+,CD25++ T cells displayed reduced antiinflam-
22 matory cytokine production and less potent suppressor capacity, since 4 times more
23 cells were required to achieve 50% inhibition compared with their membrane-bound
24 TNF α -negative counterparts. Treatment of RA patients with TNF α -specific antibodies led
25 to a reduction in the number of membrane-bound TNF α -positive CD4+,CD25++ T cells
26 from peripheral blood.

27

28 Conclusion

29 Our data indicate that the absence of membrane-bound TNF α on CD4+,CD25++ T cells
30 can be used to characterize and enrich for Treg cells with maximal suppressor potency.
31 Enrichment of membrane-bound TNF α -negative CD4+,CD25+ cells in the CD4+,CD25++
32 T cell compartment may contribute to restoring the compromised suppressive ability of
33 CD4+,CD25++ T cell populations in RA patients after anti-TNF α treatment.

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1 Introduction

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3 CD4⁺,CD25⁺ Treg cells are generated in the thymus and periphery and represent a
4 crucial regulator of peripheral self-tolerance (1;2). They are hyporesponsive to T cell
5 receptor (TCR) stimulation in vitro (3;4); however, once activated, they can suppress the
6 proliferation and cytokine production of effector T cells, antibody production of B cells,
7 and the function of monocyte/macrophages (3-6). Hence, CD4⁺,CD25⁺ Treg cells have
8 been successfully used to prevent/treat immunologic diseases in a variety of animal
9 models, such as collagen-induced arthritis (CIA) (7-9).

10 Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic
11 synovial inflammation resulting in cartilage and bone damage, eventually leading to
12 joint destruction. Several different cell types and their mediators are thought to be
13 involved in this tissue-destructive inflammation, including T cells, B cells, and proinflam-
14 matory cytokines such as tumor necrosis factor α (TNF α). The chronic inflammatory
15 process suggests that immune regulation in RA patients is disturbed. Indeed, numerous
16 reports have outlined disturbances in CD4⁺,CD25⁺ T cell functions in patients with RA
17 as well as other autoimmune diseases (10-15), suggesting an imbalance between Treg
18 cell and effector T cell activities. However, functional CD4⁺,CD25⁺ Treg cells have also
19 been reported in RA patients (16;17), which could be a consequence of the complex
20 phenotype of these cells in humans and the lack of specific cell surface markers to define
21 and isolate Treg cells.

22 Although CD25 appears to identify a relatively homogeneous population of Treg cells
23 in naive mice, the presence of significant amounts of activated conventional CD25⁺
24 T cells in humans makes it difficult to distinguish "genuine" Treg cells from activated
25 conventional T cells in the CD4⁺,CD25⁺ T cell compartment. Indeed, several studies
26 have suggested that only the CD4⁺ T cell subset expressing the highest levels of CD25
27 (termed CD25^{high} or CD25⁺⁺) is mostly forkhead box P3-positive (FoxP3⁺) and has in
28 vitro suppressive activity (18). Nonetheless, activated "conventional" T cells may still be
29 present in the CD4⁺,CD25⁺⁺ T cell subset, because FoxP3, the most commonly used
30 marker for Treg cells (19), is also transiently expressed in activated nonsuppressive T cells
31 (20-22). For this reason, the CD4⁺,CD25⁺⁺ T cell population that homogeneously ex-
32 presses FoxP3 might not consist entirely of Treg cells but could also contain conventional
33 activated CD4⁺,CD25⁺⁺ T cells, especially in situations in which chronic T cell activation
34 is thought to occur. The possible "contamination" of Treg cells with "conventional"
35 CD4⁺,CD25⁺⁺ T cells might also contribute to the compromised suppressive activity of
36 CD4⁺,CD25⁺⁺ T cell populations in autoimmune diseases such as RA (10-15), in which
37 chronic T cell activation has been implicated. Likewise, other surface markers, such
38 as CTLA-4 and glucocorticoid-induced TNF receptor, which have been reported to be
39 expressed on Treg cells (23;24), do not distinguish Treg cells from activated effector cells

1 in humans. Therefore, they cannot be used as such to identify Treg cells, especially not in
2 conditions with chronic T cell activation. Hence, further characterization and therapeutic
3 use of Treg cells would be greatly facilitated by the identification of surface markers that
4 could be used to define and isolate Treg cells with high suppressive activity.

5 TNF α is a pleiotropic cytokine that is bioactive both as a transmembrane protein and as
6 a homotrimeric secreted molecule (25). Membrane-bound TNF α is expressed on normal
7 human peripheral blood T cells after activation (26). As part of a range of proteins that
8 are expressed on the cell surface after T cell activation, membrane-bound TNF α on CD4+
9 T cells can modulate the activation of macrophages and costimulate B cells, thereby
10 enhancing their antibody production (27-29). Because it is conceivable that these lat-
11 ter features are mediated by conventional effector T cells rather than by Treg cells, and
12 given that T cells also up-regulate CD25 upon activation, we reasoned that membrane-
13 bound TNF α -expressing CD4+,CD25++ T cells are enriched for activated conventional
14 T cells rather than for naturally occurring Treg cells. In that case, membrane-bound
15 TNF α -positive CD4+,CD25++ T cells would differ in phenotype and/or functional activity
16 compared with their membrane-bound TNF α -negative counterparts.

17 In the present study, we show that the expression of membrane-bound TNF α on
18 CD4+,CD25++ T cells is correlated with disease activity in RA patients. Although
19 membrane-bound TNF α -positive and TNF α -negative CD4+,CD25++ T cells express com-
20 parable levels of FoxP3 and are both hyporesponsive to TCR stimulation, membrane-
21 bound TNF α -negative CD4+,CD25++ T cells produce more of the antiinflammatory
22 cytokine interleukin-10 (IL-10) and suppress the proliferation and cytokine production
23 of responder T cells in a more profound manner compared with their membrane-bound
24 TNF α -positive counterparts. Furthermore, treatment with anti-TNF α antibodies results
25 in a reduced frequency of membrane-bound TNF α -positive T cells in the CD4+,CD25++
26 T cell compartment in RA patients, suggesting that selective depletion of the less sup-
27 pressive membrane-bound TNF α -positive CD4+,CD25++ T cells by anti-TNF α antibodies
28 contributes to the restoration of Treg cell activity in RA patients that has been reported
29 previously (10-12;30).

31 **Patients and methods**

32 **Samples**

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34 Fresh peripheral blood was obtained from healthy adult donors with no history of auto-
35 immune diseases. Fresh peripheral blood from RA patients was obtained from patients
36 attending the outpatient clinic of the Leiden University Medical Center. All RA patients
37 fulfilled the 1987 revised classification criteria of the American College of Rheumatol-
38 ogy (formerly, the American Rheumatism Association) (31). The characteristics of these
39

1 RA patients are summarized in **Table 1**. Buffy coats were obtained from the Sanquin
2 Bloodbank (Sanquin, Amsterdam, The Netherlands). Informed consent was provided in
3 accordance with procedures approved by the local human ethics committee.

4 **Table 1.** Characteristics of and disease parameters for the RA patients*

5 Age, years	55.2 \pm 14.5
6 RA duration, years	5.55 \pm 2.79
7 DAS [†]	1.60 \pm 0.88
8 ESR, mm/hour [†]	17.6 \pm 15.6
9 No. of women/no. of men	22/18
10 No. RF positive/no. RF negative [‡]	22/5
11 No. anti-CCP-positive/no. anti-CCP negative [‡]	18/9

12 * Except where indicated otherwise, values are the mean \pm SEM. RA = rheumatoid arthritis.

13 [†] Data on the Disease Activity Score (DAS) and erythrocyte sedimentation rate (ESR) were available for 23
14 patients when membrane-bound tumor necrosis factor α (TNF α) expression was analyzed.

15 [‡] Data on rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) status were available for
16 27 patients when membrane-bound TNF α expression was analyzed.

17 Antibodies

19 Fluorescein isothiocyanate (FITC)-conjugated anti-CD25 (2A3), phycoerythrin (PE)-con-
20 jugated anti-CD25 (M-A251), anti-CD127 (hIL-7R-M21), anti-CD45RO (UCHL1), allophy-
21 cocyanin (APC)-conjugated anti-CD4 (SK3), anti-CD25 (M-A251), and peridin chlorophyll
22 protein-Cy5.5-conjugated anti-CD4 (SK3) were all from BD Biosciences (San Jose, CA).
23 PE/FITC-coupled anti-FoxP3 (PCH101; eBioscience, San Diego, CA) was used as indicated.
24 Anti-TNF α monoclonal antibody (infliximab) was labeled with FITC (Calbiochem, La Jolla,
25 CA) and used to detect the expression of membrane-bound TNF α on cells.

26 Cell isolation

28 Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood or
29 buffy coats by centrifugation over Ficoll-Hypaque gradients. CD4⁺ T cells were enriched
30 from PBMCs by negative selection with the CD4⁺ T cell isolation kit (Miltenyi Biotec,
31 Bergisch Gladbach, Germany). CD4⁺ T cells were then stained with APC-conjugated
32 anti-CD4, PE-conjugated anti-CD25, and FITC-conjugated anti-TNF α for 30 minutes at
33 4°C. After washing, CD4⁺, CD25⁻ responder T cells, membrane-bound TNF α -positive cells,
34 and membrane-bound TNF α -negative cells within the CD4⁺, CD25⁺⁺ T cell population
35 were isolated by sorting with an Aria flow sorting machine (BD Biosciences). The sorted
36 cells were gated first on small lymphocytes by forward and side scatter, then for CD4⁺
37 and CD25⁺⁺ (top 2-5% of CD4⁺ T cells) expression, and then for membrane-bound TNF α
38 expression. CD4-depleted PBMCs irradiated with 4,500 rads were used as feeders.

39

1 **Flow cytometric analysis**

2 Single-cell suspensions were prepared, and surface molecules were stained for 30
3 minutes at 4°C with optimal dilutions of each antibody. After fixation and permeabiliza-
4 tion, cells were incubated with anti-FoxP3 antibody. Expression of cell surface and in-
5 tracellular markers was assessed using flow cytometry (FACSCalibur; Becton Dickinson,
6 Mountain View, CA) after gating on live cells determined by scatter characteristics. Data
7 were analyzed by CellQuest Pro software (Becton Dickinson).

9 **Proliferation and suppression of T cells**

10 Cells were plated at 1×10^4 cells/well in 96-well plates with 1 µg/ml phytohemaggluti-
11 nin (PHA) and feeders (5×10^4 cells/well) in the presence or absence of 50 units/ml IL-2.
12 Cells were pulsed with 3H-thymidine (0.5 µCi/well) on day 4, and proliferation was as-
13 sessed 18 hours later using a liquid scintillation counter. To test for suppressive capacity,
14 CD4+,CD25- responder T cells were stimulated as described above without the addition
15 of exogenous IL-2. Autologous membrane-bound TNFα-positive CD4+,CD25++ T cells
16 or membrane-bound TNFα-negative CD4+,CD25++ T cells were added, and suppression
17 was assessed by determining 3H-thymidine incorporation as well as by measuring the
18 amounts of TNFα and interferon-γ (IFNγ) in culture supernatants.

20 **Cytokine detection**

21 To determine the concentration of cytokines, BD CBA Flex Sets (for IL-10 and TNFα) (BD
22 Biosciences) or a capture enzyme-linked immunosorbent assay (for IFNγ) was used to
23 analyze culture supernatants after 96 hours, following the protocol provided by the
24 manufacturer. The beads were analyzed on an LSRII system using BD FCAP Array soft-
25 ware (BD Biosciences).

27 **Statistical analysis**

28 The Mann-Whitney U test was used to compare Treg cell-mediated suppression results
29 and cell frequencies in healthy controls and RA patients. Paired t-tests were used to
30 compare measurements in RA patients before and after anti-TNFα treatment. Spear-
31 man's correlations were used to compare cell frequencies with subject age. All statistical
32 analyses were performed using GraphPad Prism 4.00 software (GraphPad Software, San
33 Diego, CA). *P* values less than 0.05 were considered significant.

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1 Results

2 3 Preferential expression of membrane-bound TNF α on CD4 $^{+}$,CD25 $^{++}$ T cells in 4 vivo

5 Recent findings have indicated that the suppressive function of CD4 $^{+}$,CD25 $^{++}$ T cells is
6 compromised in patients with active RA (10;11). However, the suppressive capability of
7 the CD4 $^{+}$,CD25 $^{++}$ T cell population is reversed after anti-TNF α therapy (10-12;30). Given
8 the prominent role of TNF α in inflammation and in inflammatory autoimmune diseases
9 such as RA (32-34), and given the effector functions of membrane-bound TNF α on acti-
10 vated CD4 $^{+}$ T cells (i.e., macrophage activation and increment of antibody production)
11 (27-29), it is conceivable that the membrane-bound TNF α -expressing CD4 $^{+}$,CD25 $^{++}$ T
12 cell population is enriched for "conventional" activated T cells.

13 We therefore hypothesized that this T cell population possesses inferior suppressive
14 abilities compared with the non-membrane-bound TNF α -expressing CD4 $^{+}$,CD25 $^{++}$
15 T cell population. Furthermore, we speculated that membrane-bound TNF α -positive
16 CD4 $^{+}$,CD25 $^{++}$ T cells are preferentially depleted following anti-TNF α therapy, which in
17 turn could contribute, at least partially, to the reappearance of the suppressive function
18 of isolated CD4 $^{+}$,CD25 $^{++}$ T cell populations from RA patients after anti-TNF α therapy
19 (10-12;30). Hence, we wished to examine the phenotype and function of membrane-
20 bound TNF α -expressing cells in more detail to determine whether these characteristics
21 could be used to distinguish T cells with different suppressive potency within the
22 CD4 $^{+}$,CD25 $^{++}$ T cell compartment. To this end, we first characterized membrane-bound
23 TNF α expression on freshly isolated human CD4 $^{+}$ T cells.

24 PBMCs from RA patients were purified, and membrane-bound TNF α expression was
25 detected by flow cytometry. Similar proportions of CD4 $^{+}$,CD25 $^{-}$ (mean 13.9% [range
26 3.43-30.8) and CD4 $^{+}$, CD25 $^{\text{intermediate}}$ (mean 13.3% [range 3.68-28.1]) T cells stained posi-
27 tive for membrane-bound TNF α . However, within the CD4 $^{+}$,CD25 $^{++}$ T cell compartment,
28 a significantly higher percentage of membrane-bound TNF α -positive T cells (mean
29 26.6% [range 7.49-49.2]) was observed (**Figures 1A and B**). Similar results were obtained
30 with cells isolated from healthy individuals (n = 19) (data not shown). Furthermore, a
31 consistent increase in the percentage of membrane-bound TNF α -positive T cells was
32 observed within the FoxP3 $^{+}$,CD4 $^{+}$ T cell population compared with their FoxP3 $^{-}$,CD4 $^{+}$
33 counterparts (data not shown). These results could not be explained by binding
34 of the antibody to TNF α captured by surface TNF receptors (TNFRs), because most
35 TNFRII $^{+}$,CD4 $^{+}$,CD25 $^{++}$ cells are membrane-bound TNF α negative, and, more important,
36 in line with previous reports (35), similar membrane-bound TNF α expression was ob-
37 served after preincubation of the cells with high concentrations of soluble TNF α (10 and
38 50 ng/ml) (data not shown). Together, these data indicate that human CD4 $^{+}$,CD25 $^{++}$ T
39 cells express membrane-bound TNF α .

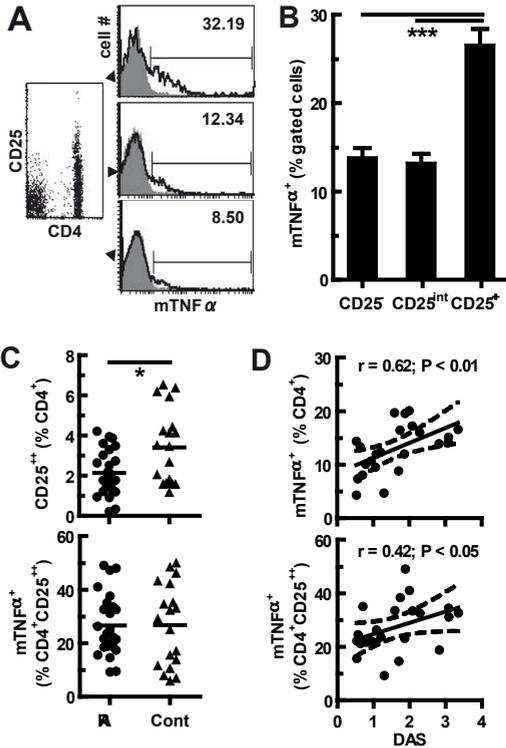


Figure 1. Correlation of membrane-bound tumor necrosis factor α (mTNF α) expression with disease activity in patients with rheumatoid arthritis (RA). Peripheral blood mononuclear cells were isolated from RA patients and stained with anti-CD4, anti-CD25, and anti-TNF α (infliximab). CD25 and membrane-bound TNF α expression was monitored by flow cytometry. All patients analyzed were naive for TNF α antagonists. **A**, Representative histograms (right panel) showing membrane-bound TNF α staining (black line) and isotype staining (shaded area) within each cell population. The gates for CD4⁺,CD25^{lo} (bottom box), CD4⁺,CD25^{intermediate} (CD4⁺,CD25^{int}; middle box), and CD4⁺,CD25^{hi} (top box) T cells are demonstrated in the left panel. Numbers within boxes in the right panel indicate the percentage of positive cells within each gate. **B**, Bar graph of the percentage of membrane-bound TNF α -expressing cells in different T cell populations. Results are expressed as the mean and SEM from 40 different individuals. ** = $P < 0.001$. **C**, Comparison of CD4⁺,CD25^{hi} or membrane-bound TNF α -expressing CD4⁺,CD25^{hi} T cells between 26 RA patients (mean \pm SEM age 46.7 ± 10.9 years) and 19 age-matched healthy controls (Cont) (mean \pm SEM age 44.3 ± 13.8 years). Horizontal lines indicate median values for each group. * = $P < 0.05$. **D**, Frequency of membrane-bound TNF α -expressing cells within CD4⁺ and CD4⁺,CD25^{hi} T cell compartments, plotted against the Disease Activity Scores (DAS) for 23 patients. Linear regression (solid line) with 95% confidence intervals (dashed lines) are shown.

Correlation of membrane-bound TNF α expression with disease activity in RA patients

We next wished to study whether the expression of membrane-bound TNF α on CD4⁺,CD25^{hi} T cells differs between healthy controls and RA patients. To this end, we compared CD25 and membrane-bound TNF α expression in 26 RA patients with that in

1 age-matched healthy controls. Although the number of CD4⁺,CD25⁺⁺ T cells decreased
2 significantly in these RA patients, there was no significant difference in the frequency of
3 membrane-bound TNF α -expressing CD4⁺,CD25⁺⁺ T cells (**Figure 1C**).

4 The Disease Activity Score (DAS) (36) was available for 23 of the 40 RA patients studied
5 at the same time that membrane-bound TNF α expression was analyzed (**Table 1**). Given
6 the role of TNF α in the pathogenesis of RA (32-34) and the expression and function of
7 membrane-bound TNF α on activated CD4⁺ T cells (25-29), we wished to address the
8 question of whether there is any association between disease activity and the expres-
9 sion of membrane-bound TNF α . As shown in **Figure 1D**, the analysis revealed that the
10 percentages of membrane-bound TNF α -positive T cells within the CD4⁺ ($r = 0.62$, $P <$
11 0.01) or CD4⁺,CD25⁺⁺ ($r = 0.42$, $P < 0.05$) T cell populations were correlated with disease
12 activity in these RA patients. Given that membrane-bound TNF α expression was also
13 associated with the erythrocyte sedimentation rate in RA patients (data not shown),
14 these data indicated that the expression of membrane-bound TNF α is associated with
15 the inflammatory immune response in RA patients. No association of its expression with
16 disease duration was observed (data not shown).

17 18 **Expression of other markers on membrane-bound TNF α -positive and TNF α -** 19 **negative CD4⁺,CD25⁺⁺ T cells**

20 Given the association of membrane-bound TNF α -expressing CD4⁺,CD25⁺⁺ T cells
21 with disease activity (Figure 1D) and the possibly compromised suppressive activity of
22 CD4⁺,CD25⁺⁺ T cells in patients with active RA, as previously reported (10;11), we wished
23 to characterize these membrane-bound TNF α -expressing CD4⁺,CD25⁺⁺ T cells in more
24 detail. Since FoxP3 is the most commonly used marker for human CD4⁺,CD25⁺ Treg
25 cells thus far (19), and because surface CD127 expression is inversely associated with
26 the presence of FoxP3 and the suppressive function of freshly isolated human CD4⁺ T
27 cells from healthy donors (37), we first determined how surface membrane-bound TNF α
28 expression correlates with FoxP3 and CD127 expression. Therefore, PBMCs were stained
29 for CD4, CD25, and membrane-bound TNF α , together with either FoxP3 or CD127.

30 As shown in **Figure 2**, no significant difference in intracellular FoxP3 expression
31 was observed between membrane-bound TNF α -positive and membrane-bound
32 TNF α -negative CD4⁺,CD25⁺⁺ T cells, either in the percentage of positive cells or in
33 the expression levels within each cell compartment. Likewise, a similar percentage of
34 CD127^{-/low} cells was observed within the membrane-bound TNF α -negative CD4⁺,CD25⁺⁺
35 and membrane-bound TNF α -positive CD4⁺,CD25⁺⁺ T cell subsets. Because the expres-
36 sion of CD45RO, the memory marker for human T cells, has been used to discriminate
37 different CD4⁺,CD25⁺ Treg cell populations (38), we analyzed the expression of this
38 marker on membrane-bound TNF α -positive and TNF α -negative CD4⁺,CD25⁺⁺ T cells
39 as well. Almost all membrane-bound TNF α -positive CD4⁺,CD25⁺⁺ T cells displayed

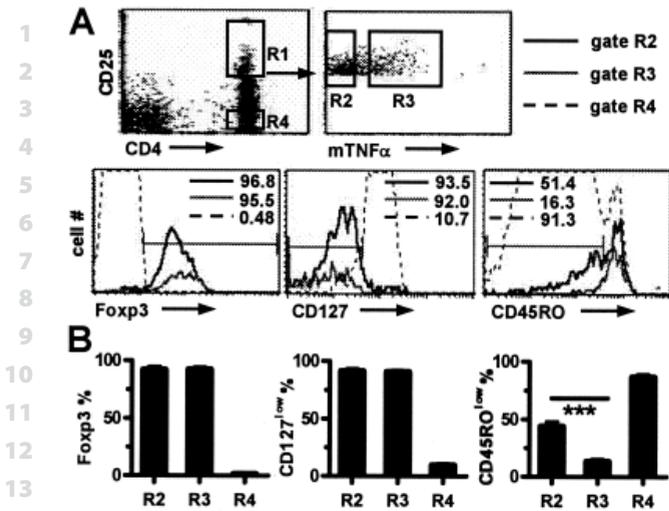


Figure 2. Expression of other markers on membrane-bound tumor necrosis factor α (mTNF α)-positive and mTNF α -negative CD4⁺, CD25⁺⁺ T cells. Peripheral blood mononuclear cells were stained with anti-CD4, anti-CD25, and anti-TNF α , together with anti-CD127, anti-CD45RO, or anti-forkhead box P3 (anti-FoxP3) (intracellular for FoxP3). A, Gating for histograms was performed on the basis of CD4, CD25, and membrane-bound TNF α expression, as indicated in the upper panel. Membrane-bound TNF α -negative CD4⁺, CD25⁺⁺ (region 2 [R2]) and membrane-bound TNF α -positive CD4⁺, CD25⁺⁺ (R3) T cell subsets were gated by first restricting to CD4⁺, CD25⁺⁺ T cells (R1). Percentages of FoxP3⁺, CD127^{-low}, and CD45RO^{-low} cells within each gate are shown in the lower panel. CD4⁺, CD25⁻ T cells (R4) were included as a control. B, Bar graph shows the percentages of FoxP3⁺, CD127^{-low}, and CD45RO^{-low} cells within each gate as shown in A. Results are expressed as the mean and SEM from 8 different individuals. *** = $P < 0.001$.

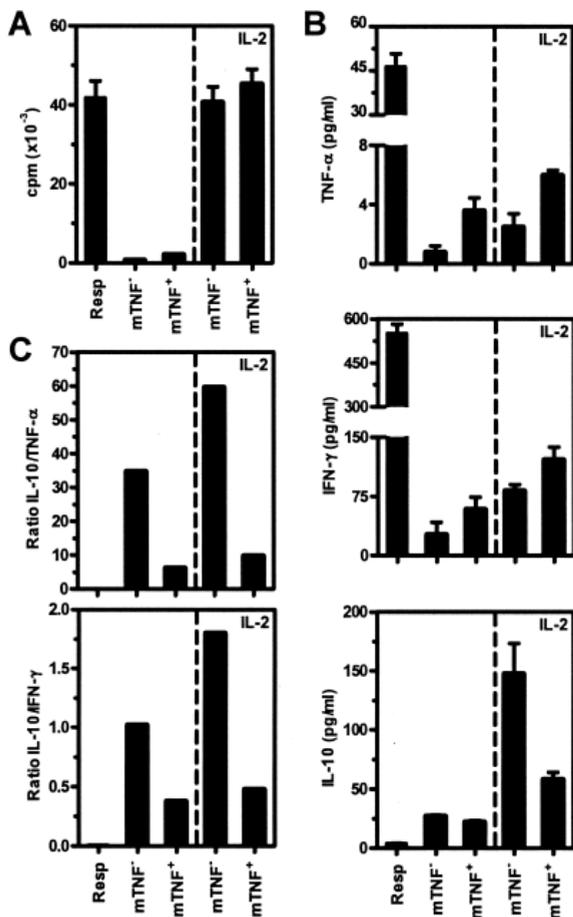
high expression of CD45RO, whereas a significant proportion of their membrane-bound TNF α -negative counterparts had lower levels of CD45RO expression (Figure 2). Similar results were obtained when membrane-bound TNF α -positive or TNF α -negative CD4⁺, CD25⁺ T cells were analyzed (data not shown). Together, these data indicate that membrane-bound TNF α expression on CD4⁺, CD25⁺⁺ T cells correlates with levels of CD45RO expression, despite similar FoxP3 and CD127 expression.

Association of membrane-bound TNF α with decreased production of antiinflammatory cytokines in CD4⁺, CD25⁺⁺ T cells

Human CD4⁺, CD25⁺⁺ Treg cells are hyporesponsive and produce relatively high levels of the antiinflammatory cytokine IL-10 but low levels of proinflammatory cytokines such as TNF α and IFN γ (39). To analyze whether membrane-bound TNF α expression on CD4⁺, CD25⁺⁺ T cells discriminates cells with differential cytokine production profiles, we purified membrane-bound TNF α -positive CD4⁺, CD25⁺⁺ and membrane-bound TNF α -negative CD4⁺, CD25⁺⁺ T cells from buffy coats using fluorescence-activated cell

1 sorting (FACS), and we subsequently stimulated them with PHA and autologous feeders
 2 in the presence or absence of exogenous IL-2.

3 With respect to proliferation and production of the proinflammatory cytokines TNF α
 4 and IFN γ , both membrane-bound TNF α -positive and membrane-bound TNF α -negative



31 **Figure 3.** Association of membrane-bound tumor necrosis factor α (mTNF α) with decreased production
 32 of antiinflammatory cytokines in CD4⁺, CD25⁺⁺ T cells. Fluorescence-activated cell-sorted CD4⁺, CD25-
 33 responder T cells (Resp), membrane-bound TNF α -negative CD4⁺, CD25⁺⁺ T cells (mTNF⁻), and
 34 membrane-bound TNF α -positive CD4⁺, CD25⁺⁺ T cells (mTNF⁺) (1×10^4 cells/well) were activated with
 35 phytohemagglutinin (1 μ g/ml) and feeders (5×10^4 cells/well) in the absence or presence of 50 units/ml
 36 exogenous interleukin-2 (IL-2). The sorting gates for membrane-bound TNF α -negative and membrane-
 37 bound TNF α -positive CD4⁺, CD25⁺⁺ T cells are depicted in the upper panel of Figure 2A. A, Cell
 38 proliferation was determined by 3H-thymidine uptake 5 days later. B and C, Culture supernatants were
 39 collected after 96 hours, and the amounts of TNF α , interferon- γ (IFN γ), and IL-10 produced (B) or the ratios
 of IL-10:TNF α and IL-10:IFN γ (C) were determined. Results are expressed as the mean and SEM from 3 or 4
 triplicate cultures and are representative of those from 4 independent experiments.

1 CD4+,CD25++ T cells were hyporesponsive compared with autologous CD4+,CD25- re-
2 sponder T cells (**Figures 3A and B**). Nonetheless, when membrane-bound TNF α -positive
3 and TNF α -negative CD4+,CD25++ cells were compared side by side, membrane-bound
4 TNF α -positive CD4+,CD25++ T cells produced more TNF α and IFN γ (Figure 3B) and
5 displayed a higher rate of proliferation (Figure 3A). In contrast, both CD4+,CD25++ T
6 cell populations produced more IL-10 upon stimulation compared with CD4+,CD25- T
7 cells (Figure 3B). Intriguingly, however, membrane-bound TNF α -negative CD4+,CD25++
8 T cells produced twice the amount of IL-10 in comparison with their membrane-bound
9 TNF α -positive counterparts after stimulation in the presence of IL-2 (Figure 3B). The
10 mean \pm SEM IL-10 level produced by membrane-bound TNF α -negative CD4+,CD25++
11 T cells was 121.99 ± 14.27 pg/ml compared with 51.64 ± 7.68 pg/ml produced by their
12 membrane-bound TNF α -positive counterparts after 4 days of stimulation in the pres-
13 ence of IL-2 ($P < 0.05$; $n = 4$) (data not shown).

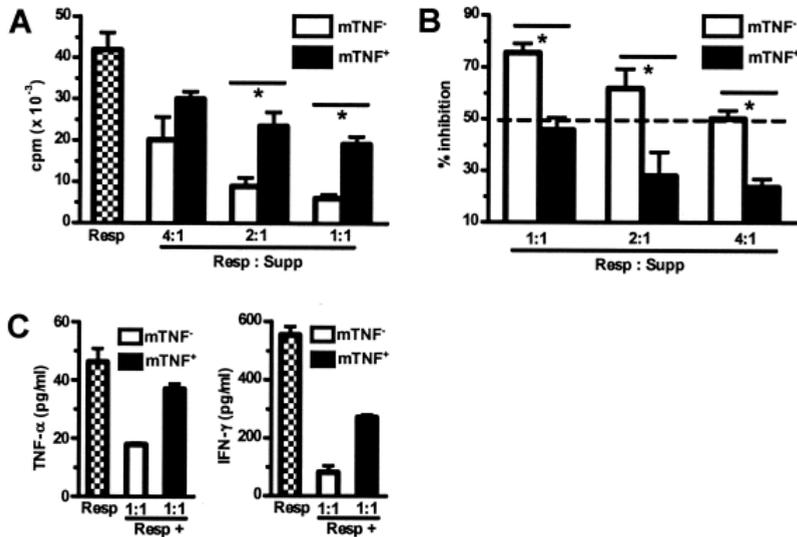
14 These results indicate a difference in functional capacity between these two
15 CD4+,CD25++ T cell subsets, which is also illustrated by the ratio of IL-10 to TNF α or
16 IFN γ (**Figure 3C**). Together, these data point to the possibility that membrane-bound
17 TNF α -negative CD4+,CD25++ T cells display a greater suppressive potency compared
18 with their membrane-bound TNF α -expressing counterparts.

19 20 **Membrane-bound TNF α on CD4+,CD25++ T cells distinguishes a population** 21 **with enhanced suppressor activity**

22 To address whether differential membrane-bound TNF α expression is indeed as-
23 sociated with differential suppressive activity, fluorescence-activated cell-sorted
24 membrane-bound TNF α -positive CD4+,CD25++ and membrane-bound TNF α -negative
25 CD4+,CD25++ T cells were placed in a standard in vitro suppression assay using
26 CD4+,CD25- T cells as responder T cells. As shown in **Figure 4A**, membrane-bound
27 TNF α -negative CD4+,CD25++ T cells suppressed proliferation more robustly than did
28 membrane-bound TNF α -positive CD4+,CD25++ T cells. Approximately 4-fold fewer
29 membrane-bound TNF α -negative CD4+,CD25++ cells than membrane-bound TNF α -
30 positive CD4+,CD25++ cells were required to achieve 50% suppression (**Figure 4B**).

31 To further confirm the differential suppressive capability between membrane-bound
32 TNF α -positive and TNF α -negative CD4+,CD25++ cells, we next analyzed the amounts
33 of TNF α and IFN γ in the coculture supernatants. Consistent with the results described
34 above, the addition of membrane-bound TNF α -negative CD4+,CD25++ T cells at a 1:1
35 ratio resulted in a stronger reduction of TNF α and IFN γ production than the addition of
36 membrane-bound TNF α -positive CD4+,CD25++ T cells (**Figure 4C**). This difference could
37 not be attributed to the higher cytokine production by membrane-bound TNF α -positive
38 CD4+,CD25++ T cells, since they secreted relatively small amounts of TNF α and IFN γ
39 under these conditions (Figure 3B). Taken together, these observations indicate that

1 membrane-bound TNF α -negative CD4 $^{+}$,CD25 $^{++}$ T cells possess an enhanced suppressive activity.
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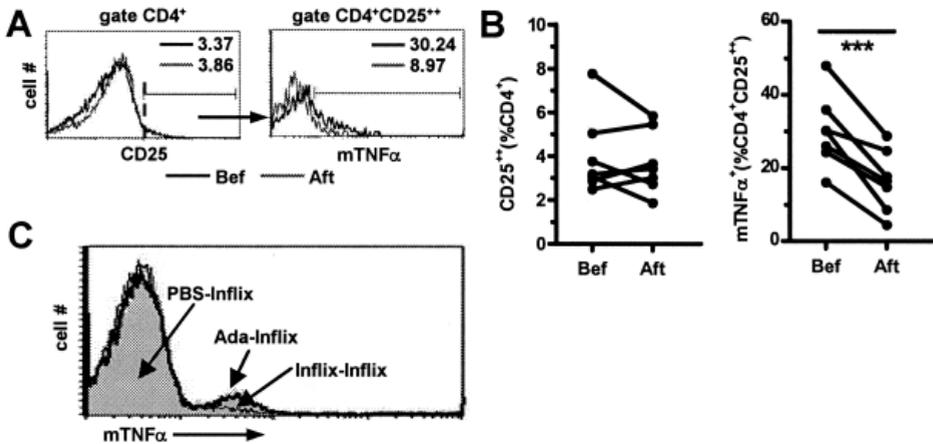
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 18 **Figure 4.** Membrane-bound tumor necrosis factor α (mTNF α) on CD4 $^{+}$,CD25 $^{++}$ T cells distinguishes a
 19 population with enhanced suppressor activity. Fluorescence-activated cell-sorted CD4 $^{+}$,CD25 $^{-}$ responder
 20 T cells (Resp) (1×10^4 cells/well) were activated with phytohemagglutinin ($1 \mu\text{g/ml}$) and feeders (5×10^4
 21 cells/well) in the absence or presence of membrane-bound TNF α -negative CD4 $^{+}$,CD25 $^{++}$ suppressor
 22 T cells (Supp) or membrane-bound TNF α -positive CD4 $^{+}$,CD25 $^{++}$ suppressor T cells (mTNF $^{-}$ or mTNF $^{+}$,
 23 respectively) at a 4:1, 2:1, or 1:1 (Resp:Supp) ratio. In all experiments, CD4 $^{+}$,CD25 $^{-}$ responder T cells were
 24 also added at a 1:1 ratio as a control (i.e., 2×10^4 responder T cells per well), which always yielded greater
 25 proliferation and cytokine production (data not shown). A, Proliferation after 5 days was determined by
 26 ^3H -thymidine incorporation. B, The average percent inhibition of proliferation is shown. The dashed line
 27 indicates 50% inhibition. C, Culture supernatants were collected after 96 hours, and the amounts of TNF α
 28 and interferon- γ (IFN γ) were analyzed in cultures of responders only (Resp) or responders plus suppressors
 29 (Resp +) at a 1:1 ratio. Results are expressed as the mean and SEM from 3 or 4 cultures and represent (A
 30 and C) or summarize (B) 4 independent experiments with different individuals. * = $P < 0.05$.

30 Decreased numbers of membrane-bound TNF α -positive T cells in RA patients 31 after anti-TNF α treatment

32 Previous studies have shown that anti-TNF α therapy could reverse the compromised
 33 function of isolated CD4 $^{+}$,CD25 $^{++}$ T cells in RA patients (10-12;30). Given our observa-
 34 tions that membrane-bound TNF α -positive CD4 $^{+}$,CD25 $^{++}$ T cells bear a less potent sup-
 35 pressor activity (Figure 4) and are associated with disease activity in RA patients (Figure
 36 1D), we wished to know whether the expression of membrane-bound TNF α on T cells
 37 was decreased in RA patients after TNF α -blocking therapy (adalimumab; $n = 7$).

38 All these patients responded well to anti-TNF α treatment, as evidenced by a reduction
 39 in the mean \pm SEM DAS in 28 joints (40), from 7.06 ± 0.56 to 3.89 ± 0.66 three months

1 after treatment ($P < 0.0001$) (data not shown). There was no difference in the absolute
 2 number and the frequency of CD3+ T cells, CD4+ T cells, and CD4+,CD25++ T cells in
 3 peripheral blood (**Figures 5A and B** and data not shown) before and 3 months after
 4 adalimumab treatment. However, a significantly decreased expression of membrane-
 5 bound TNF α on CD4+,CD25++ T cells (Figures 5A and B) as well as on total CD3+ or CD4+
 6 T cells (data not shown) was observed in all patients. This could not be explained by the
 7 presence of adalimumab on membrane-bound TNF α -positive T cells in vivo that would
 8 influence the detection of membrane-bound TNF α expression ex vivo by infliximab,
 9 since the same expression levels of membrane-bound TNF α were observed on cells
 10 regardless of whether or not they had been preincubated with adalimumab (**Figure 5C**).



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26 **Figure 5.** Decreased expression of membrane-bound tumor necrosis factor α (mTNF α) in patients with
 27 rheumatoid arthritis (RA) after anti-TNF α treatment. Seven patients with active RA (Disease Activity Score
 28 in 28 joints >5) were evaluated for CD25 and membrane-bound TNF α expression both before and 3
 29 months after anti-TNF α therapy (40 mg adalimumab subcutaneously every other week in combination
 30 with stable doses of methotrexate [7.5-25 mg/week orally]). A, Representative histograms showing CD25
 31 staining on CD4+ T cells or membrane-bound TNF α expression on CD4+,CD25++ T cells in RA patients
 32 before (Bef) and 3 months after (Aft) adalimumab treatment. Numbers indicate the percentage of
 33 CD25++ expression on CD4+ T cells (left panel) or membrane-bound TNF α expression on CD44+,CD25++
 34 T cells (right panel). B, Summary of the percentage of CD25++ T cells within CD4+ T cells or of the
 35 frequency of CD4+,CD25++ T cells coexpressing membrane-bound TNF α in RA patients before and 3
 36 months after TNF α -blocking therapy. Each dot represents 1 patient ($n = 7$). C, Histogram showing lack
 37 of effect of adalimumab on the detection of membrane-bound TNF α by fluorescein isothiocyanate
 38 (FITC)-labeled infliximab. Cells were incubated with phosphate buffered saline (PBS-Inflix; shaded area),
 39 adalimumab (Ada-Inflix; thick black line), or infliximab (Inflix-Inflix; thin black line) at 4°C for 30 minutes.
 After washing, cells were stained for membrane-bound TNF α expression by FITC-labeled infliximab. ***=
 $P < 0.001$.

1 Discussion

2
3 Immunosuppressive CD4⁺,CD25⁺⁺ Treg cells are of great interest for immunotherapy
4 to prevent transplant rejection and for the treatment of autoimmune diseases. Success-
5 ful treatment of mice with CIA, an animal model for human RA, by adoptive transfer of
6 CD4⁺,CD25⁺ Treg cells has been reported (9). However, unlike the situation in mice, in
7 which expression of CD25 identifies a relatively homogeneous population of Treg cells,
8 the identification and manipulation of Treg cells in humans is hampered due to the pres-
9 ence of large amounts of activated conventional T cells within the CD4⁺,CD25⁺ T cell
10 compartment. Although the highest expression of CD25 (termed CD25^{high} or CD25⁺⁺)
11 on CD4⁺ T cells is now widely used to identify, isolate, and characterize naturally occur-
12 ring Treg cells in humans, the reported defective suppressive function of the isolated
13 CD4⁺,CD25⁺⁺ T cell population from patients with autoimmune diseases, such as RA,
14 might still be attributed, at least in part, to the heterogeneous composition of this T cell
15 population (10-15). Therefore, one of the main obstacles to therapeutic applications of
16 Treg cells is to define and isolate cells displaying high inhibitory activity.

17 TNF α is a pleiotropic cytokine critical for inflammation, maintenance of secondary
18 lymphoid organ structure, and host defense against various pathogens as well as for
19 the pathogenesis of RA (32-34;41;42). Moreover, membrane-bound TNF α expressed on
20 the surface by CD4⁺ T cells activates macrophages, costimulates B cell activation, and
21 increases antibody production (27-29). Therefore, we speculated that membrane-bound
22 TNF α -expressing CD4⁺,CD25⁺⁺ T cells are enriched for activated conventional T cells
23 and/or T cells with inferior suppressive function, since Treg cells inhibit the function of
24 monocyte/macrophages, reduce antibody production of B cells, and produce very small
25 amounts of TNF α , even in the presence of IL-2 (5;6;21). Our results reveal a significant
26 expression of membrane-bound TNF α within the CD4⁺,CD25⁺⁺ T cell population both
27 in RA patients and in healthy individuals (Figure 1 and data not shown). Although
28 membrane-bound TNF α -positive CD4⁺,CD25⁺⁺ T cells display suppressive activity, this
29 activity is much less potent than that of their membrane-bound TNF α -negative coun-
30 terparts (Figure 4). Therefore, we conclude that membrane-bound TNF α expression is
31 a marker that is inversely associated with the suppressive capacity of CD4⁺,CD25⁺⁺ T
32 cells.

33 It has been shown that the surface expression of CD127 is inversely correlated with
34 the expression of FoxP3 and the suppressive function of fresh human CD4⁺ T cells
35 (37). However, our data indicate that these 2 markers are not differentially expressed
36 by CD4⁺,CD25⁺⁺ T cells that do and those that do not express membrane-bound
37 TNF α (Figure 2). Although FoxP3 was thought to program the development and function
38 of murine CD4⁺,CD25⁺ Treg cells (19), FoxP3 is not a Treg cell-specific marker in humans,
39 since it is also expressed in activated effector T cells (20-22;43). Likewise, not all of the

1 CD4+,CD127-/^{low} cells in human PBMCs are FoxP3+, since CD127 is down-regulated in
2 recently activated effector T cells (22;37). Therefore, our data suggest that the expres-
3 sion levels of FoxP3 or CD127 do not correlate with the suppressive activity *within* the
4 CD4+,CD25++ T cell compartment, whereas the absence of membrane-bound TNF α
5 on CD4+,CD25++ T cells can be used to characterize and enrich Treg cells with maximal
6 suppressor potency.

7 Recent reports have suggested that *in vivo* a portion of the human CD4+,CD25++ T
8 cell population is generated from rapidly dividing, highly differentiated memory CD4+ T
9 cells (44;45). Since the memory T cell marker CD45RO is highly expressed on membrane-
10 bound TNF α -positive CD4+,CD25++ T cells (Figure 2), and membrane-bound TNF α
11 expression on these cells correlates with disease activity (Figure 1D), it is tempting to
12 speculate that these FoxP3+, anergic membrane-bound TNF α -positive CD4+,CD25++
13 T cells are "adaptive" Treg cells derived from memory T cells in the periphery. Such cells
14 could emerge as a negative feedback to dampen immune responses, as proposed previ-
15 ously (44-47). Our results showing that a subset of CD4+,CD25++ T cells display a less
16 potent suppressive capacity and bear additional activation markers, such as CD45RO,
17 would support this notion. The reason why such cells also express membrane-bound
18 TNF α could relate to their former function as effector cells, during which they secreted
19 TNF α . The expression of membrane-bound TNF α would, in that context, be a residual
20 property of these cells. This could also explain the correlation between their presence
21 and disease activity, since it is conceivable that greater disease activity would lead to
22 more exhausted effector T cells that express membrane-bound TNF α .

23 Treatment of RA patients with TNF α blockers results in significant clinical benefit,
24 which most likely involves the inhibition of the TNF α -induced inflammatory cytokine
25 cascades (34). Moreover, recent studies have suggested that anti-TNF α antibodies could
26 also reverse the "compromised" suppressive function of isolated CD4+,CD25++ T cells
27 in RA patients (10-12;30). This restoration is thought to involve the neutralization of
28 circulating soluble TNF α that inhibits Treg cell function via TNFR II and/or the induction
29 of a distinct CD62L-,CD25++ Treg cell population via transforming growth factor β in RA
30 patients (11;12). Considering the fact that adalimumab could deplete membrane-bound
31 TNF α -expressing cells via inducing apoptosis, antibody-dependent cellular cytotoxicity,
32 and/or complement-dependent cytotoxicity both *in vitro* and *in vivo* (48-50), our results
33 provide an additional explanation for the reversal of Treg cell activity of the isolated
34 CD4+,CD25++ T cell population after anti-TNF α antibody therapy, since they indicate
35 that these antibodies deplete the less suppressive membrane-bound TNF α -positive
36 CD4+,CD25++ T cells from the CD4+,CD25++ T cell population in RA patients (Figures
37 4 and 5).

38 In conclusion, we have demonstrated that membrane-bound TNF α is expressed on a
39 significant percentage of CD4+,CD25++ T cells in human peripheral blood, and that this

1 expression is correlated with disease activity in RA patients. Moreover, the absence of
2 surface membrane-bound TNF α expression can be used to identify and isolate a subset
3 of CD4 $^{+}$,CD25 $^{++}$ T cells with potent suppressive capability. Furthermore, our results
4 indicate that, in addition to blocking soluble circulating TNF α and/or inducing a new
5 Treg cell population (11;12), selective depletion of the less effective membrane-bound
6 TNF α -positive suppressors from the CD4 $^{+}$,CD25 $^{++}$ T cell compartment in RA patients
7 may be another explanation for the recovery of Treg cell function by anti-TNF α therapy.

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CHAPTER 9

Functional regulatory immune responses against human cartilage glycoprotein-39 in health vs. proinflammatory responses in rheumatoid arthritis

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1 Abstract

2
3 The class of immune response against autoantigens could profoundly influence the
4 onset and/or outcome of autoimmune diseases. Until now, there is only limited in-
5 formation on the antigen-specific balance between proinflammatory and regulatory
6 responses in humans. Here we analyzed the natural immune response against a candi-
7 date autoantigen in rheumatoid arthritis, human cartilage glycoprotein-39 (HC gp-39).
8 Peripheral blood mononuclear cells from healthy individuals reacted against HC gp-39
9 with the production of IL-10 but not IFN- γ . Ex vivo assays indicated that the naturally
10 occurring HC gp-39-specific immune response in bulk is powerful enough to suppress
11 antigen-specific recall responses, demonstrating that rather than being unresponsive,
12 the HC gp-39-directed immune response in healthy individuals shows a strong bias
13 toward a regulatory phenotype. Moreover, CD4+ T cell lines directed against HC gp-
14 39 expressed CD25, glucocorticoid-induced tumor necrosis factor receptor, and Foxp3
15 molecules and were capable of suppressing antigen-specific T cell responses. Cell-cell
16 contact was required for this suppression. As opposed to healthy individuals, the HC
17 gp-39-directed immune response in 50% of patients with rheumatoid arthritis exhibits
18 polarization toward a proinflammatory T helper 1 phenotype and is significantly less
19 powerful in suppressing antigen-specific recall responses. Together these findings in-
20 dicate that the presence of HC gp-39-specific immune responses in healthy individuals
21 may have an inhibitory effect on inflammatory responses in areas where HC gp-39 is
22 present. Furthermore, these data indicate that the class of HC gp-39-directed immune
23 response in rheumatoid arthritis patients has shifted from an antiinflammatory toward
24 a proinflammatory phenotype.

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1 Introduction

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3 Rheumatoid arthritis (RA) is a chronic inflammatory disease with a variable disease
4 outcome, which is characterized by an inflammatory process of unknown origin in
5 multiple joints. Although the pathogenesis of RA is multifactorial, RA susceptibility and/
6 or severity is strongly associated with the presence of isotype-switched autoantibodies
7 and certain HLA class II alleles. These observations clearly point to a role for T cells in
8 the pathological processes underlying RA. Therefore, much interest has focused on the
9 phenotype of the T cell response that is present in the inflamed joints of RA patients. In
10 general, these T cells display a typical T helper (Th)1-like phenotype (1). These T cells,
11 either directly or indirectly, contribute to the proinflammatory cascades and inflamma-
12 tory environment that is observed within the joints of RA patients (2). In contrast, other
13 mechanisms are likely to be involved in the protection against autoimmunity such as
14 the production of antiinflammatory cytokines such as IL-10 and TGF- β . In this respect, a
15 population of naturally occurring T cells, T regulatory (Treg) cells, have regained much
16 interest in the last decade. These cells display antiinflammatory and antiproliferative
17 functions. In several animal model systems, it has been shown that Treg cells play a criti-
18 cal role in the generation and maintenance of tolerance and generally inhibit immune
19 responses that are potentially deleterious to the host (3). Several T cell subsets have
20 been described that mediate these protective immunoregulatory effects, of which the
21 most extensively studied T cell subset is characterized by the expression of CD4 and
22 CD25 in both mice and humans (4–6).

23 Investigations into the role of Treg cells have been difficult because no specific marker
24 for Treg cells has been identified so far. The expression of CD25, cytotoxic T lymphocyte
25 antigen-4, the tumor necrosis factor (TNF) family molecule glucocorticoid-induced TNF
26 receptor (GITR), and Foxp3 have been used to identify Treg cells but, in general, Treg
27 cells are characterized on the basis of a functional definition through their ability to
28 down-regulate other immune responses (7, 8).

29 *In vitro* studies indicate that T cell receptor triggering by, for example, anti-CD3 is
30 required for Treg cell function (3, 9), indicating that antigen recognition most likely
31 represents the natural trigger initiating Treg cell activity. However, there is only limited
32 evidence for autoantigen-specific regulatory T cell populations in humans. Nonetheless,
33 knowledge on the autoantigens capable of inducing regulatory circuits in humans is
34 important because this knowledge can be used for the design of rationally defined vac-
35 cines that prevent and/or inhibit autoimmune diseases. Studying the question whether
36 a certain antigen can induce regulatory circuits in humans has been proven difficult
37 because of various technical aspects related to low precursor frequencies and the in-
38 herent difficulty of expanding cells with a regulatory phenotype *in vitro*. Moreover, the
39 choice of the antigenic stimulus is particularly important and, in case a certain antigen

1 is selected for further studies, high purity to avoid immune activation by contaminants,
2 such as lipopolysaccharide, within the antigen preparation is essential.

3 Here, we have studied the natural immune responses against human cartilage glyco-
4 protein-39 (HC gp-39), a candidate autoantigen in RA. HC gp-39 is a major constituent
5 of human cartilage and is overexpressed in synovial specimens and cartilage from RA
6 patients (10). It has been shown that HC gp-39 is efficiently processed and presented
7 in the context of MHC class II molecules by dendritic cells (DCs) and macrophages (11,
8 12). HC gp-39 can also serve as a target for T cells, because HC gp-39-specific MHC class-
9 II-restricted T cell responses can readily be induced in HLA-DR4-transgenic mice (13).
10 Moreover, proliferative responses have been described in RA patients, indicating that
11 HC gp-39-directed T cells are present also in humans (13–15). Interestingly, intranasal
12 treatment with HC gp-39 suppressed murine collagen-induced arthritis (16). These
13 observations indicate that HC gp-39 can activate/boost regulatory circuits that interfere
14 with arthritis in case the antigen is presented in an environment that is adapted to
15 induce tolerogenic responses to innocuous antigens. Moreover, they indicate that HC
16 gp-39-directed T cells can escape thymic deletion and that HC gp-39-specific T cells are
17 present in the natural T cell repertoire. Together, these observations identify HC gp-39
18 as an interesting human autoantigen and have led us to hypothesize that HC gp-39 is
19 driving regulatory responses in humans. Therefore, the aim of this work was to deter-
20 mine whether HC gp-39 could stimulate regulatory or suppressive immune responses
21 in healthy individuals and whether the bias of HC gp-39-directed immunity is altered in
22 RA patients.

23 24 25 **Materials and methods**

26 27 **Blood Samples and HLA-DR Typing**

28 After informed consent, citrated or heparinized venous blood was collected from
29 healthy blood bank donors and RA patients classified as having definite RA according to
30 the 1987 American College of Rheumatology criteria for RA (17). The characteristics of
31 the RA patients are summarized in **Table 1**. Peripheral blood mononuclear cells (PBMCs)
32 were isolated and used directly or were cryopreserved in liquid nitrogen. HLA-DR typ-
33 ing was performed on genomic DNA by using the sequence specific oligonucleotide
34 (PCR-SSO) as described in refs. 18 and 19. In this study, DRB1*04-positive donors were
35 selected for further analysis.

36 37 **Antigens**

38 Clinical-grade human recombinant HC gp-39 (Org 39141; 10 µg/ml final conc.) was
39 kindly provided by N.V. Organon, Oss, The Netherlands. The recall-response antigen

Table 1. Characteristics of RA patients

No of patients tested	43
Age, years	53.7 ± 13.9 (24.0-78.5)*
Male/female	15/28
HLA-DR4	43/43
Disease duration, years	6.8 ± 1.8 (3.3-9.7)*
Erosive disease	34/43 (81%)
RF positive	33/43 (76.7%)
DMARD treatment	40/43 (93%)
More than one DMARD in use	11/43 (25.6)
More than one DMARD used in the past	30/43 (69.8%)
Methotrexate treatment	23/43 (53.5%)
Prednisolone treatment	3/47 (7.0%)

RF, rheumatoid factor-IgM (RF positive if ≥5 units; DMARD, disease modifying antirheumatic drug.

*Expressed as mean ± SD (range, in parentheses).

mix consisted of a mixture of tetanus toxoid (T.tox) (0.75 limit of flocculation unit/ml final conc.; National Institute of Public Health and the Environment, Bilthoven, The Netherlands), purified protein derivative (PPD) from *Mycobacterium tuberculosis* (5 µg/ml final conc.; Statens Serum Institute Copenhagen), and *Candida albicans* (0.005%, HAL Allergen Lab, Haarlem, The Netherlands).

Keyhole limpet hemocyanin (KLH; 10 µg/ml final conc.) was obtained from Pierce Biotechnology. Phytohaemagglutinin (PHA HA16; 2 µg/ml final conc.) was obtained from Remel (Lenexa, KS). Clinical-grade human insulin (Actrapid; 10 µg/ml final conc.) was obtained from Novo Nordisk, Bagsvaerd, Denmark. The preferentially expressed antigen of melanoma (PRAME)-derived peptide PRA¹⁴²⁻¹⁵¹ (SLYSFPEPEA; 5 ng/ml final conc.) was synthesized by solid-phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422, Langenfeld, Germany) and subsequently stored at -20°C until usage.

Cytokine Elispot Analysis

PBMCs were stimulated with antigen in culture medium supplemented with 10% FCS for 4 days in 24-well plates. Thereafter, the cells were seeded in 4 replicate wells at a density of 10⁵ cells per well (IFN-γ) or 1.5 × 10⁵ cells per well (IL-10) of a MultiScreen 96-well plate (Millipore), coated with IFN-γ or IL-10 catching antibody (MABTECH, Natcha, Sweden). After overnight incubation at 37°C, the plates were developed according to the manufacturer's procedures. The number of visible spots was counted in a BioReader 3000 (BioSys, Karben, Germany). To examine the nature of the responder cells, PBMCs were cultured as described above or depleted of CD3+ T cells (>97% pure). The CD3-negative fraction was seeded at a density of 5 × 10⁴ cells per well. For statistical analysis of differences between cocultures in the inhibition assays, Student's *t* test was used.

1 Differences in IFN- γ production between healthy donors and RA patients were analyzed
2 by the Mann–Whitney U test.

3

4 **Detection of Antigen-Specific IL-10-Secreting Cells**

5 Fresh PBMCs were stimulated with antigen in culture medium supplemented with 10%
6 autologous or AB-serum for 4 days in 24-well plates. Thereafter, the cells were labeled
7 with anti-IL-10 antibody (Miltenyi Biotec, Amsterdam) and subsequently seeded over-
8 night in six-well plates. Next, the IL-10 secreting cells were stained according to the
9 manufacturer's procedures and analyzed on a FACSCalibur (Becton Dickinson). Isotype-
10 matched mouse IgG1-phycoerythrin-conjugated control mAb (clone X40) was obtained
11 from Becton Dickinson.

12

13 **Generation of T Cell Lines**

14 PBMCs were stimulated *in vitro* (2×10^6 per ml) with PPD (5 $\mu\text{g/ml}$), T.tox. (0.75 limit of
15 flocculation unit/ml) or HC gp-39 (10 $\mu\text{g/ml}$). After 4–5 days, cells were expanded for
16 11–13 days in culture medium supplemented with 10% FCS and recombinant human
17 IL-2 (rhIL-2; 25 units/ml; Chiron) alone [T cell line raised against PPD from *Mycobacterium*
18 *tuberculosis* (T_{PPD})/T cell line raised against T.tox. ($T_{\text{T.tox}}$)] or rhIL-2 combined with rhIL-15
19 (10 units/ml; TEBU BIO Peprotech, Heerhugowaard, The Netherlands) for the T cell line
20 raised against HC gp-39 ($T_{\text{HCgp-39}}$).

21

22 **Proliferation Assays**

23 Proliferation of T cell lines was measured in 96-well plates in 4–6 replicate cultures. To
24 analyze putative toxic properties of HC gp-39, 2.5×10^3 cells from T_{PPD} or $T_{\text{T.tox}}$ were incu-
25 bated per well together with PPD or T.tox and 5×10^3 irradiated (3,000 rad) autologous
26 PBMCs as antigen-presenting cells (APCs) with or without HC gp-39. No inhibition of
27 proliferation was detected in T_{PPD} or $T_{\text{T.tox}}$, indicating that HC gp-39 was nontoxic. For
28 the inhibition assays, each well contained 10^4 T_{PPD} cells, 10^4 autologous $T_{\text{HCgp-39}}$ cells, $2 \times$
29 10^4 irradiated autologous PBMCs as APCs, and antigen. Control antigen clinical grade
30 insulin (Actrapid) was heat pretreated to disrupt 3D structures and prevent putative
31 cell interactions. After 4 days of culturing, proliferation was determined by overnight
32 incorporation of [^3H]thymidine. For statistical analysis the two-tailed Student's t test for
33 unpaired samples was used.

34

35 **Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE)-Labeled Cell Division**

36 **Analysis**

37 T_{PPD} or $T_{\text{T.tox}}$ were labeled with 0.5 μM CFSE (Molecular Probes). The CFSE-labeled cells ($5 \times$
38 10^5) were incubated with 10^6 irradiated PBMCs as APCs, antigen, and with or without $5 \times$
39 10^5 unlabeled cells from an autologous $T_{\text{HCgp-39}}$. After 3 days, the CFSE content of the T_{PPD}

1 was analyzed on a FACSCalibur. To quantify the absolute number of divided CFSE-labeled
2 cells and to correct for apoptotic or dead CFSE-labeled cells, the number of analyzed
3 cells was standardized by the addition of 15×10^3 Flow-Count fluorospheres per sample
4 (Beckman Coulter). Acquisition was stopped when 3×10^3 beads were counted.

5 In the transwell experiments, $T_{\text{HCgp-39}}$ (5×10^5) or control cell line $T_{\text{T.tox}}$ were cultured in
6 the inner wells (24-well plate) in medium containing 5×10^5 irradiated PBMCs as APCs
7 with or without HC gp-39 protein. Equal numbers of CFSE-labeled T_{PPD} cells and APCs
8 were added into the outer wells in the same medium with or without PPD. After 4 days
9 of culture, the CFSE content of the T_{PPD} was analyzed on a FACSCalibur.

10 11 **Recall Suppression Assays**

12 PBMCs were incubated with or without HC gp-39 in a 24-well plate. After 3–5 h, the
13 cultures were supplemented with a recall-response antigen mix (see above). After 4
14 days of culturing, the overnight IFN- γ excretion of the culture was analyzed by elispot
15 (see above). Differences in the HC gp-39-induced changes in IFN- γ excretion between
16 healthy donors and RA patients were analyzed by the Mann–Whitney *U* test.

17 18 **Cytotoxic T Lymphocyte (CTL) Inhibition Assays**

19 An HLA-A2-restricted CTL clone directed against a tumor-associated antigen PRAME-
20 derived peptide was a kind gift from J. Kessler (Leiden University Medical Center). The
21 isolation, maintenance, and properties of the CTL clone are described in ref. 20. During
22 stimulation with the PRAME peptide, this CTL clone produces high amounts of IFN- γ . For
23 inhibition assays, PBMCs from an HLA-A2-positive donor known to secrete IL-10 after
24 incubation with HC gp-39 were incubated with or without HC gp-39 protein. After 48 h,
25 the PRAME peptide together with 30,000 A2-restricted PRAME-specific CTLs was added.
26 The overnight IFN- γ excretion of the coculture was analyzed by elispot (see above).

27 28 29 **Results**

30 31 **HC gp-39-Specific Immune Reactivity Is Characterized by IL-10 Production**

32 Because various observations indicate the presence of HC gp-39-directed T cells in the
33 human T cell repertoire, combined with the finding that presentation of HC gp-39 in
34 a noninflammatory/tolerogenic environment can induce regulatory circuits (16), we
35 wished to evaluate the natural HC gp-39-directed immune response in healthy individu-
36 als. As elispot analyses allow the detection of low precursor frequencies with preserva-
37 tion of the class of immune responsiveness (e.g., as measured by IFN- γ or IL-10 produc-
38 tion), we used elispot analyses after a short, 4-day culture of PBMCs with antigens. Our
39 data show that the PBMCs from 17 of 31 healthy donors analyzed reacted against HC

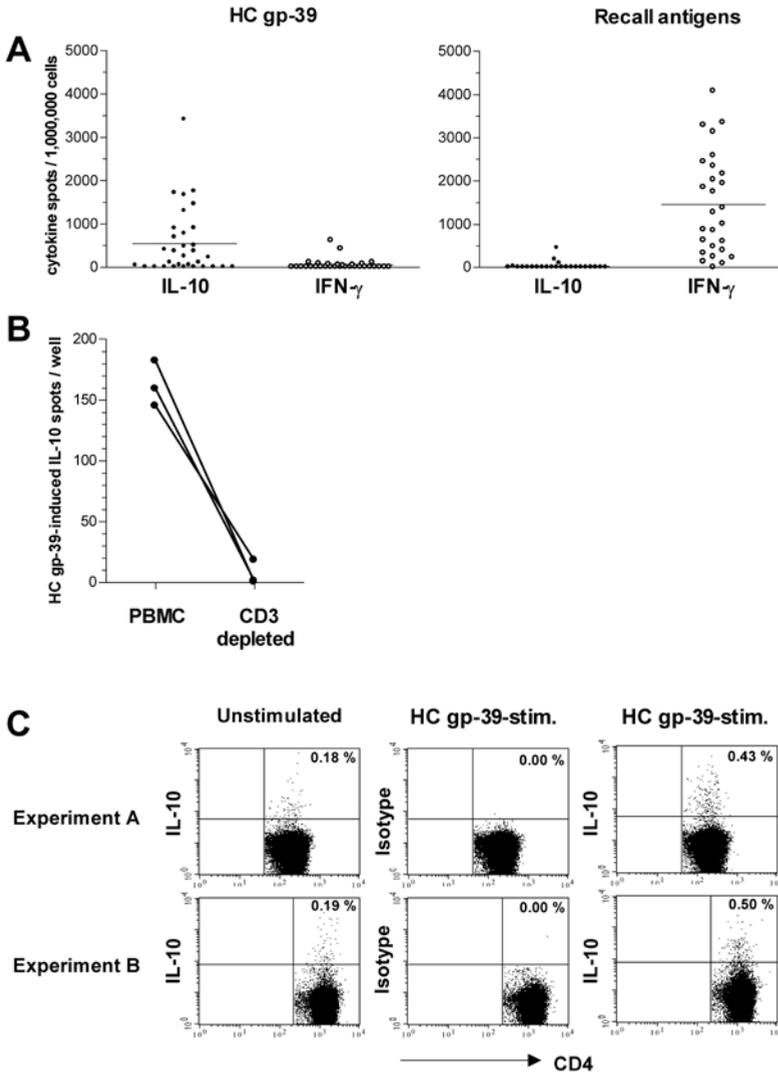


Figure 1. HC gp-39-specific immune reactivity in healthy donors. (A) PBMCs from healthy donors ($n = 31$) react against HC gp-39 by production of IL-10 but not IFN- α (Left). Responses induced by “control” recall antigens are dominated by IFN- γ production (Right). (B) HC gp-39-induced IL-10 responses are mediated by T cells. Shown in an analysis of PBMCs from three different donors for IL-10 responses to HC gp-39, in which HC gp-39 was cultured with unmanipulated PBMCs or PBMCs immunomagnetically depleted of CD3+ T cells before elispot analysis. Background responses (medium alone) were subtracted from HC gp-39 responses. (C) CD4+ T cells respond to HC gp-39 with the production of IL-10. Unstimulated CD4+ T cells (Left) produce background amounts of IL-10 (0.2%), whereas IL-10 was produced by 0.4–0.5% of HC gp-39-stimulated CD4+ T cells (Right). As a control, HC gp-39-stimulated CD4+ T cells (Center) were stained with an isotype-matched control mAb (0.0% staining).

1 gp-39 with the production of IL-10 without the concomitant production of IFN- γ (**Figure**
 2 **1A Left**). This response is specific for HC gp-39 because responses toward a mix of typical
 3 Th1-associated recall antigens are dominated by IFN- γ production (**Figure 1A Right**).
 4 Importantly, HC gp-39 was not toxic to the cells, because relatively high doses of HC gp-
 5 39 did not suppress the proliferative capacity of defined T cell lines (data not shown). No
 6 IL-10 responses could be detected anymore after depletion of CD3+ cells from the PBMC
 7 cultures stimulated with HC gp-39 (**Figure 1B**). In contrast, IL-10 responses remained in
 8 CD3-depleted cultures stimulated with phytohemagglutinin. Together, these findings
 9 indicate that T cells or CD3+ natural killer cells are responsible for the IL-10 production
 10 after stimulation of PBMCs with HC gp-39. To further validate the source of IL-10 produc-
 11 tion after HC gp-39-stimulation, we investigated whether CD4+ cells produce IL-10 in
 12 response to HC gp-39 by flow cytometry. Although we were not able to detect IL-10
 13 production toward HC gp-39 in most donors by this technique (presumably as a result
 14 of a lower sensitivity of this assay compared with elispot analyses), we reproducibly de-
 15 tected CD4+ IL-10+ cells in a donor harboring a high frequency of cells reacting against
 16 HC gp-39. As shown in **Figure 1C**, IL-10 was produced by 0.4–0.5% of CD4+ cells (Right),
 17 whereas unstimulated PBMCs produced only background amounts of IL-10 (0.2%) (Left).
 18 Together, these results indicate that the natural immune response against HC gp-39 in
 19 healthy donors is hallmarked by the production of IL-10 mediated by HC gp-39-directed
 20 T cells.

21

22 Phenotypic and Immunological Characterization of CD4+ T Cell Lines Against 23 HC gp-39

24 The observation that CD3+ T cells produce IL-10 and not IFN- γ on exposure to HC gp-39
 25 prompted us to investigate whether HC gp-39-responsive T cells represent T cells with
 26 immunoregulatory properties. For this purpose, we generated T cell lines against HC
 27 gp-39 ($T_{\text{HCgp-39}}$). $T_{\text{HCgp-39}}$ were expanded by HC gp-39-specific stimulation in the presence
 28 of IL-2 and IL-15 (21, 22) and subsequently analyzed for the presence of various markers
 29 that are associated with CD4+ T_{reg} cells. Phenotypic analyses of the generated $T_{\text{HCgp-39}}$
 30 showed that $T_{\text{HCgp-39}}$ were CD4+ expressing high levels of CD25 and glucocorticoid-
 31 induced tumor necrosis factor receptor that produced increased levels of IL-10, tumor
 32 necrosis factor α , and IFN- γ on stimulation with HC gp-39 (data not shown). Moreover,
 33 real-time PCR revealed that the $T_{\text{HCgp-39}}$ also expressed relatively high levels of Foxp3,
 34 a transcription factor highly expressed in CD4+ CD25+ T_{reg} cells (23, 24). Control Th1
 35 cell lines against PPD and T.tox. that were derived from the same donors displayed
 36 similar markers, although the level of expression was often considerably lower (data not
 37 shown). These results indicate that the phenotype of the T cell lines against HC gp-39
 38 is compatible with the molecular makeup typically found on naturally occurring CD4+
 39 CD25+ T_{reg} cells.

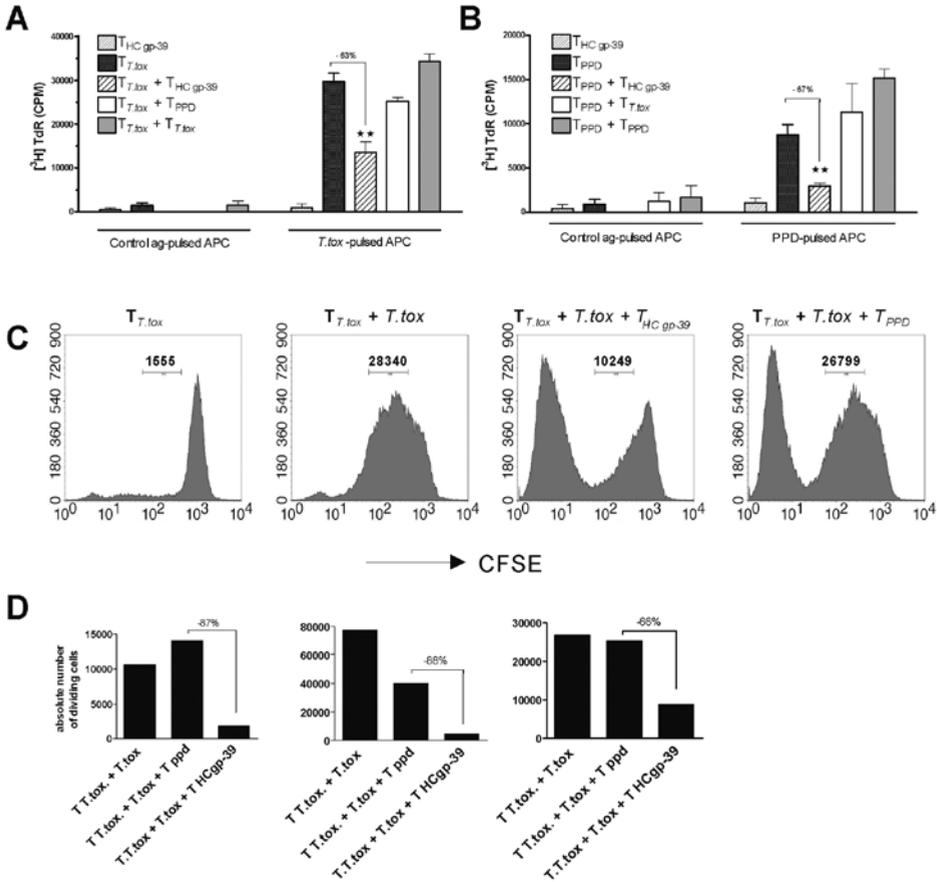


Figure 2. CD4⁺T cell lines raised against HC gp-39 ($T_{HCgp-39}$) are suppressive. (A and B) $T_{HCgp-39}$ suppresses proliferation of autologous T cell lines specific for recall antigens, $T_{T.tox}$ (A) and T_{PPD} (B). Background responses (unstimulated $T_{T.tox}$ and T_{PPD}) were subtracted from stimulated $T_{T.tox}$ and T_{PPD} . **, $P < 0.01$. (C) $T_{HCgp-39}$ can inhibit cell division of autologous CFSE-labeled $T_{T.tox}$. $T_{T.tox}$ (Far Left) was incubated with $T_{T.tox}$ -pulsed APCs alone (Center Left) or together with $T_{HCgp-39}$ (Center Right) or T_{PPD} (Far Right). The absolute number of dividing cells is indicated in each histogram. Similar results were obtained in four donors. (D) $T_{HCgp-39}$ can inhibit proliferation of autologous $T_{T.tox}$ and T_{PPD} . T cell lines directed against $T_{T.tox}$, PPD, and HC gp-39 were generated from three different donors (Left, Center, and Right). Although some suppression of $T_{T.tox}$ proliferation was sometimes observed after addition of T_{PPD} (center bar in Center), a pronounced suppressive activity is displayed by $T_{HCgp-39}$. These data are representative of nine donors analyzed. Similar data were obtained when T_{PPD} was used as a readout, adding $T_{T.tox}$ and $T_{HCgp-39}$ as suppressors (data not shown). Background responses (unstimulated $T_{T.tox}$ and T_{PPD}) were subtracted from $T_{T.tox}$ and PPD-stimulated $T_{T.tox}$ and T_{PPD} , respectively.

Nonetheless, more definitive evidence of CD4⁺ T_{reg} cells resides in the demonstration that these cell lines can inhibit proliferation of conventional CD4⁺ T cells in functional assays. Therefore, we set out to determine the regulatory capacities of HC gp-39-directed T cells in suppression assays. To this end, $T_{HCgp-39}$ cells were titrated into cultures consisting

1 of T_{PPD} or $T_{T.tox}$. The proliferative responses of T.tox-activated $T_{T.tox}$ cell lines (**Figure 2A**)
 2 and PPD-activated T_{PPD} cell lines (**Figure 2B**) were suppressed ($P < 0.01$) by the addition
 3 of $T_{HCgp-39}$ by up to 67%. In contrast, addition of T.tox-directed T cells to PPD-directed
 4 T cells and vice versa did not result in inhibition of proliferation, indicating that the
 5 suppression observed after adding $T_{HCgp-39}$ was not because of competition for space
 6 or nutrients at high cell concentrations. Moreover, inhibition required the presence of
 7 $T_{HCgp-39}$ cells because APCs pulsed with the HC gp-39 protein were not able to mediate
 8 suppression (data not shown). HC gp-39-directed T cell lines did not display detectable
 9 proliferative responses toward HC gp-39 (data not shown), which is in line with their
 10 regulatory phenotype. Together, these findings indicate that HC gp-39 by itself is not
 11 able to polarize the APCs into a “regulatory” APC and, more importantly, indicate that HC
 12 gp-39-directed T cell lines display regulatory functions.

13 The suppressive capacity of HC gp-39-directed T cell lines was confirmed in a read-
 14 out system where CFSE-labeled T.tox or PPD-directed T cell lines were cocultured with
 15 autologous HC gp-39-directed T cells. In this manner, the inhibition of proliferation of
 16 antigen-specific T cell lines can be monitored directly in a quantitative manner. **Figure**
 17 **2C** shows that addition of $T_{HCgp-39}$ results in the inhibition of $T_{T.tox}$ cell division by 62% (from
 18 28×10^3 to 10×10^3 divided cells), whereas no such inhibition was observed by adding
 19 the “crowding” control T_{PPD} . In **Figure 2D**, the results of three independent experiments
 20 are shown employing T cell lines obtained from three different donors. Although we also
 21 observed inhibition of the proliferative response after addition of an irrelevant control
 22 cell line in one of three cases, the addition of $T_{HCgp-39}$ resulted in a considerably stronger
 23 inhibition.

24 The observation that $T_{HCgp-39}$ displays a regulatory phenotype led us to investigate
 25 whether cell–cell contact was required for HC gp-39-directed T cell lines to inhibit other
 26 CD4+ effector T cells. The suppressive action of $T_{HCgp-39}$ on CFSE-labeled cell division was
 27 abrogated when cell–cell contact between $T_{HCgp-39}$ and CFSE-labeled $T_{T.tox}$ or T_{PPD} was pre-
 28 vented in transwell assays (**Figure 3 A–D**). As the control, a simultaneously performed
 29 coculture assay was performed in which strong suppression of the proliferative activity
 30 of CFSE-labeled T.tox- or PPD-directed T cell lines was observed (**Figure 3 E and F**), indi-
 31 cating that the $T_{HCgp-39}$ cells were viable and immunosuppressive. Together these results
 32 indicate that $T_{HCgp-39}$ cells are able to mediate suppression in a cell–cell contact-dependent
 33 fashion.

34

35 **Ex Vivo HC gp-39-Directed Immune Responses Can Suppress Recall Responses** 36 **and CD8+ T Cell Activity**

37 As described above, our *in vitro* assays indicate that $T_{HCgp-39}$ once activated, down-
 38 regulate other immune responses in a cell–cell contact-dependent manner. To address
 39 the question of whether the HC gp-39-reactive immune response directly after cells are

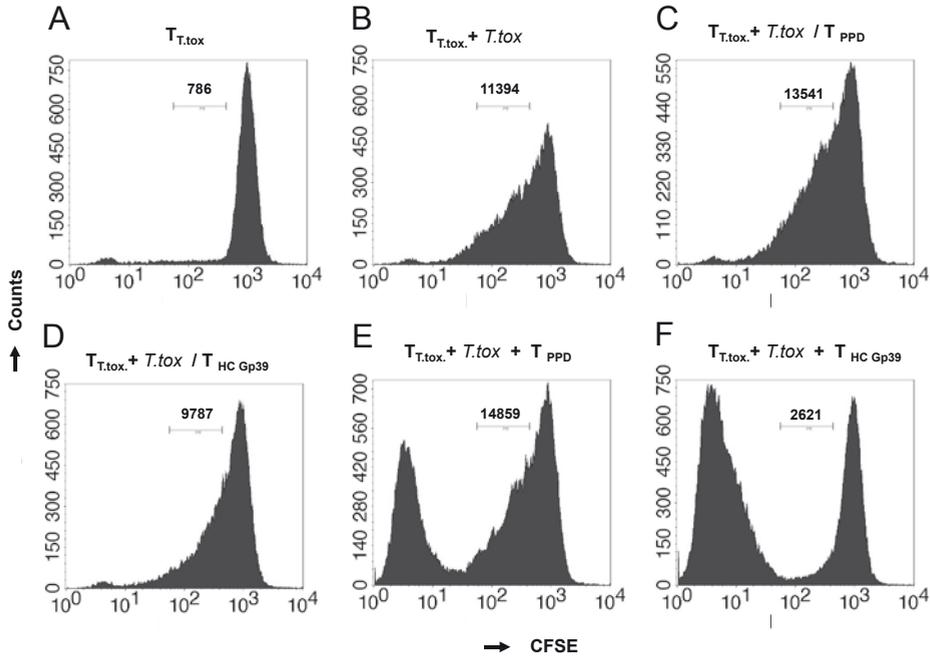


Figure 3. Cell–cell contact is required for the immunosuppressive activity of CD4⁺T cell lines directed against HC gp-39. The absolute number of dividing CFSE-labeled $T_{T.tox}$ cells is indicated in each histogram. (A) Unstimulated $T_{T.tox}$ in outer wells (B) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells. (C) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells and TPPD in inner wells. (D) $T_{T.tox}$ stimulated with T.tox-pulsed APCs in outer wells and $T_{HCgp-39}$ in inner wells. (E) $T_{T.tox}$ stimulated with T.tox-pulsed APCs and T_{PPD} together in outer wells. (F) $T_{T.tox}$ stimulated with T.tox-pulsed APCs and $T_{HCgp-39}$ together in outer wells.

taken from the donors is efficient enough to suppress other immune responses, donors that displayed IL-10 responses after stimulation with HC gp-39 were selected for further study. PBMCs from these donors were stimulated with a 10 \times diluted mixture of recall antigens to induce intermediate recall antigen-specific IFN- γ responses as measured by elispot analyses. To study whether stimulation by HC gp-39 can suppress this IFN- γ production, we triggered the PBMCs simultaneously with HC gp-39 to activate regulatory cells and the recall antigen mixture to activate recall antigen-specific memory cells. HC gp-39 could stimulate regulatory circuits as observed by a marked inhibition of the recall response (**Figure 4A**).

Next, we determined whether HC gp-39 stimulation could also inhibit CD8⁺ T cell responsiveness. Therefore, an HLA-A2-restricted CTL clone directed against a peptide derived from the tumor antigen PRAME was used. PBMCs from HLA-A2⁺ donors were stimulated with HC gp-39 and, after 48 h, loaded with the PRAME peptide. Subsequently, the PRAME-specific CTL clone was added, and IFN- γ production was measured by elispot analyses. HC gp-39-stimulated HLA-A2⁺ PBMCs were able to suppress IFN- γ production

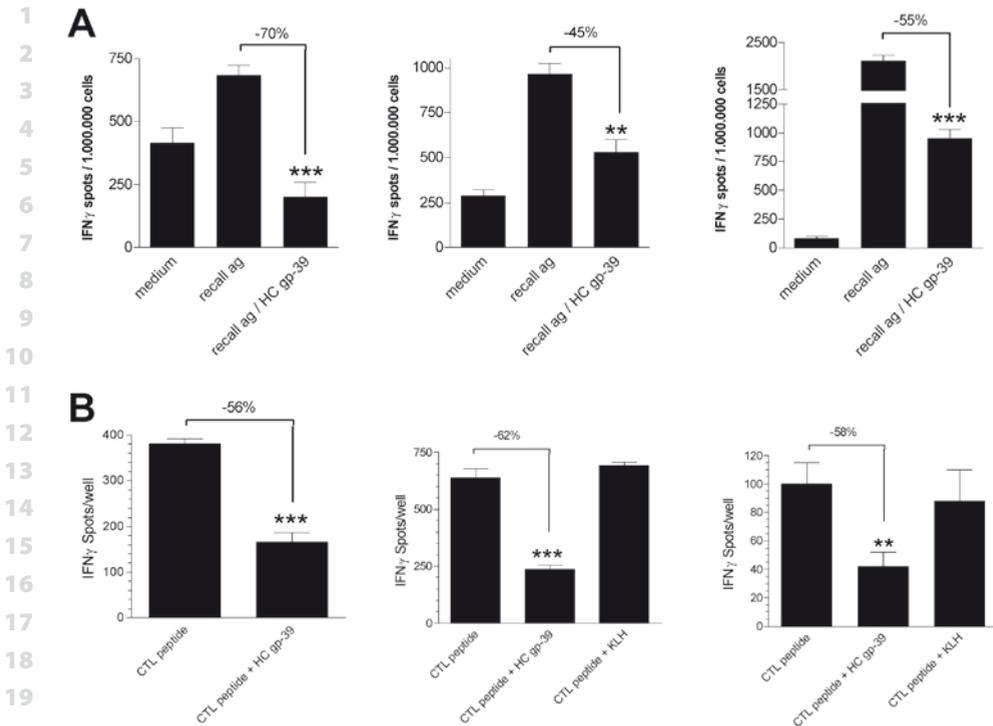


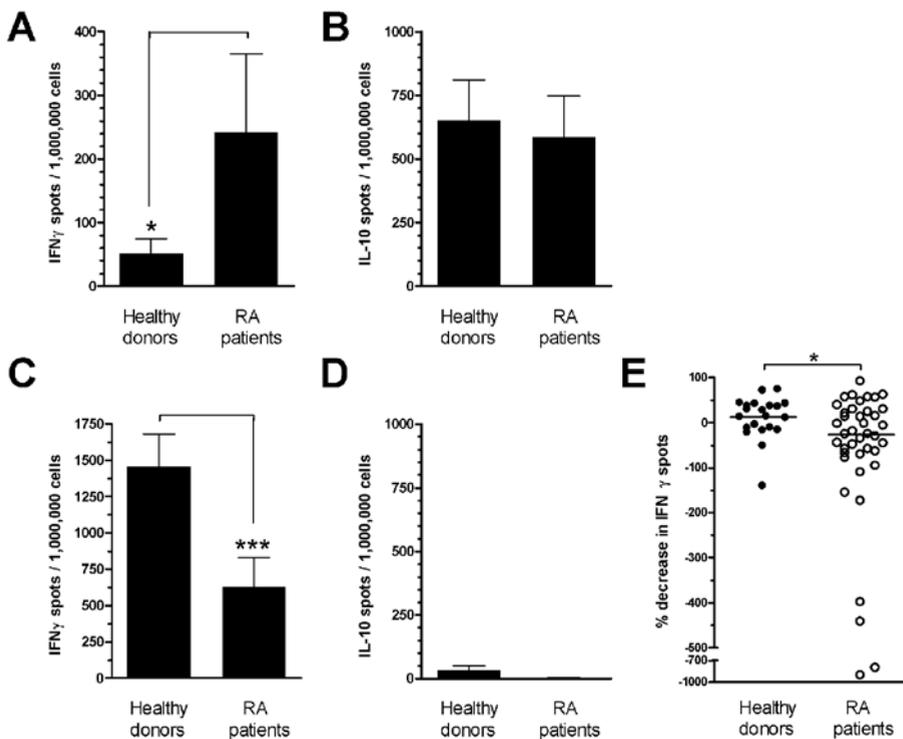
Figure 4. *Ex vivo* HC gp-39-directed immune responses can suppress other immune responses. (A) HC gp-39-activated PBMCs are able to suppress IFN-γ responses to recall antigens in three different healthy donors. (B) HC gp-39- stimulated HLA-A2+ PBMCs from three different healthy donors are capable of suppressing IFN-γ production of activated HLA-A2+ CTLs. **, $P < 0.005$; ***, $P < 0.0005$.

of activated PRAME-specific CTL as shown in **Figure 4B**. Together these data indicate that the “naturally” occurring HC gp-39-reactive immune response is highly effective in suppressing other immune responses.

The HC gp-39-Directed Immune Response in RA Patients Is Biased Toward a Proinflammatory Response

Intrigued by the antiinflammatory properties of HC gp-39-directed immune responses in healthy donors, we postulated that the immune response in RA patients might have shifted toward a proinflammatory response. Therefore, the immune response in RA patients was evaluated by employing IFN-γ and IL-10 elispots. In contrast to healthy donors, which produce primarily IL-10 (**Figs. 1 A and 5 A and B**), RA patients produce considerable amounts of IFN-γ ($P < 0.05$; i.e., 17 of 34 patients analyzed produced IFN-γ). These findings could not be attributed to a generalized increase in IFN-γ production in RA patients because the recall antigen-specific immune reactivity is diminished in RA patients compared with controls (**Figure 5 C and D**). Next we compared PBMC cultures

1 from RA patients (n = 40) with the ability of PBMC cultures from healthy donors (n = 21)
 2 to reduce recall antigen responses after costimulation with HC gp-39. It was found that
 3 HC gp-39 was less effective in stimulating regulatory response in PBMC cultures from RA
 4 patients compared with cultures from healthy controls (**Figure 5E**) because the majority
 5 of PBMC cultures from RA patients gave rise to more IFN- γ spots after costimulation
 6 with HC gp-39. In some patients, this increase was even 4-fold or higher. Together these
 7 data imply that a disease-associated bias is displayed by the HC gp-39-directed immune
 8 response.



33 **Figure 5.** IFN- γ and IL-10 responses to HC gp-39 and recall antigens by PBMCs from healthy donors (n =
 34 31) and RA patients (n = 34) as determined by elispot. (A and B) Healthy donors produce primarily IL-10
 35 but not IFN- γ , whereas RA patients produce both IL-10 and considerable amounts of IFN- γ in response to
 36 HC gp-39 (*, $P < 0.05$). (C and D) Recall antigen-directed reactivity is characterized by IFN- γ production
 37 but not IL-10 production in both RA patients and healthy donors. Moreover, PBMCs from healthy donors
 38 produce more IFN- γ after exposure to recall antigens as compared with RA patients (***, $P < 0.001$). (E) HC
 39 gp-39-stimulated PBMCs from healthy donors (n = 21; unbiased for HC gp-39-induced IL-10 production)
 are better suppressors of recall antigen-induced IFN- γ production than HC gp-39-stimulated PBMCs from
 RA patients (n = 40). *, $P < 0.03$. Line represents the median in percent decrease per group.

1 These findings indicate that HC gp-39 serves as an autoantigen in both healthy donors
2 and RA patients. However, despite a reduced Th1 response against recall antigens, the
3 HC gp-39-directed immune responsiveness in RA patients is shifted toward a proinflam-
4 matory phenotype in a substantial number of RA patients as more IFN- γ is produced and
5 is paralleled by a reduced suppression of recall responses.

6 7 8 **Discussion** 9

10 In the present study, we demonstrated a clear discrepancy in immune reactivity
11 between healthy donors and RA patients against the naturally occurring autoantigen
12 HC gp-39. IFN- γ production by PBMC cultures from the majority of healthy donors that
13 were stimulated by recall antigens could be inhibited by costimulation by HC gp-39. In
14 contrast, the majority of such cultures from RA patients displayed an increase in IFN- γ -
15 producing cells. Moreover, in contrast to HC gp-39-activated cells from RA patients that
16 also produced IFN- γ , the HC gp-39-directed immune response of healthy individuals was
17 hallmarked by the production of IL-10. IL-10 production depended on CD3-expressing
18 cells, and CD4+ T cell lines against HC gp-39 displayed regulatory capacities.

19 These findings clearly indicate that healthy individuals, rather than being nonrespon-
20 sive, frequently react to HC gp-39 with the production of IL-10. Our data can best be
21 explained by the presence of HC gp-39-directed T_{reg} cells in healthy subjects because
22 these cells have the potential to effectively suppress various proliferative and inflamma-
23 tory responses.

24 The observations that the immune response of a considerable number of RA pa-
25 tients displays a proinflammatory polarization toward HC gp-39 and is less capable of
26 inhibiting other immune responses are intriguing. Although some caution is required
27 in interpreting these results because the lack of detecting suppression does not neces-
28 sarily indicate the absence of suppressive responses, these data suggest that the inflam-
29 matory processes accompanying RA development/progression favor the induction of
30 HC gp-39-directed Th1 cells. To become activated, naïve T cells have to encounter their
31 specific antigens presented by HLA molecules on DCs. Although this event alone causes
32 naïve T cell division, it does not necessarily establish a productive immune response
33 because full mobilization to effector cells crucially depends on proper maturation of
34 DCs (25). It is plausible that the inflammatory environment observed in the joints of
35 RA patients meets the requirements for proper maturation of local DCs, enabling the
36 induction of Th1 cell responses. Moreover, it has been reported that DCs present in the
37 synovial tissue are able to present HC gp-39-derived peptides in the context of HLA
38 class II molecules (11, 12). Therefore, we hypothesize that the emergence of IFN- γ -
39 producing HC gp-39 directed T cells in RA patients is the result of HC gp-39 presentation

1 by matured DCs that have the capacity to activate Th1 cells as a consequence of the
2 maturation signals present in the inflamed synovium. In this way, the HC gp-39-specific
3 Th1 cells are a result, rather than a cause, of the disease and could also be present in
4 other (rheumatic) diseases.

5 The observation that a disease-associated bias is present with respect to the class of
6 HC gp-39-directed immune response might point toward a more generalized phenom-
7 enon because it has recently been shown also that in diabetes and multiple sclerosis
8 the autoreactive T cell response is polarized toward a proinflammatory Th1 phenotype,
9 whereas a regulatory response is observed in health (26, 27). Although these observa-
10 tions have been made in different diseases, together these findings clearly indicate that
11 induction of autoimmunity not only requires the emergence of pathogenic T cells (Th1
12 and/or Th2 cells) but also is associated with the loss of T_{reg} cell function that normally
13 protects against autoimmunity. These findings are highly relevant for the development
14 of antigen-specific intervention protocols because they suggest an approach for con-
15 trolling immune responses. Particularly, RA might represent an autoimmune disease
16 that is especially suited for targeting T_{reg} cells because, due to its systemic nature, a
17 systemic approach is required to dampen the inflammatory reaction. It is likely that T_{reg}
18 cells will focus on areas of inflammation, such as the inflamed joint, because they carry
19 several receptors for inflammatory chemokines (5, 28–30). Indeed, the presence of T_{reg}
20 cells in the inflamed joint, in addition to activated potentially pathogenic T cells, was
21 recently demonstrated in synovial fluid of RA patients (31). Moreover, we have shown
22 that $CD4^+ CD25^+ T_{reg}$ cells are also involved in collagen-induced arthritis because deple-
23 tion of $CD4^+ CD25^+$ T cells significantly increased severity and incidence of the disease,
24 whereas reconstitution studies showed that infusion of $CD4^+ CD25^+ T_{reg}$ cells conferred
25 disease protection (32). The challenge for the future will be to induce and/or expand
26 residual antigen-specific T_{reg} cell activity to treat autoimmune diseases in an antigen-
27 specific manner.

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31
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CHAPTER 10

Summary and discussion

1 Summary and discussion

2 3 Results in short

4 In this thesis several studies are described that include both clinical- and biological aspects in the field of undifferentiated (**part I**) and rheumatoid arthritis (**part II**). **Chapter**
5 **2** describes the incidence and progression of undifferentiated arthritis to rheumatoid
6 arthritis as monitored in different early arthritis clinics. Depending on the study population 6-55% of the patients who present with undifferentiated arthritis actually fulfill
7 the criteria for rheumatoid arthritis as defined by the ACR in 1987 over time. For the
8 prognosis of the first 4 years, it does not make a difference whether patients present
9 with the clinical syndrome of an evident rheumatoid arthritis or with undifferentiated
10 arthritis progressing to rheumatoid arthritis within one year (**chapter 3**). Radiographic
11 joint damage, disease activity and HAQ had a comparable course as measured over four
12 years. The difference was not explicable by the duration of symptoms and thus time of
13 the clinical phase, as median symptom duration was the same for both groups. Unfortunately, part of the window of opportunity might have been missed in the group of
14 patients that presented with undifferentiated arthritis. In the PROMPT study, patients
15 with undifferentiated arthritis were treated with either methotrexate or placebo. In the
16 MTX group, the progression to rheumatoid arthritis was postponed, and radiographic
17 damage was retarded. In patients with ACPAs the results were even more pronounced
18 (**chapter 4 and 7**). In PROMPT patients who had low and intermediate pretreatment
19 ACPA-levels and were treated with MTX, the incidence of rheumatoid arthritis was lower
20 than in patients with high levels. In a total group of DMARD-naive patients with undifferentiated arthritis or recently diagnosed rheumatoid arthritis, low and intermediate
21 pretreatment ACPA-levels were associated with a more favorable response to MTX
22 compared to patients with high levels of ACPA (**chapter 7**). The treatment in patients
23 with undifferentiated arthritis in PROMPT was aimed at a DAS <2.4, a scoring system that
24 had been developed for patients with rheumatoid arthritis. Further analysis revealed
25 that the use of DAS is justified in patients with undifferentiated arthritis (**Chapter 5**).
26 To identify which patient with undifferentiated arthritis has actually or will progress to
27 rheumatoid arthritis and will thus benefit from early DMARD treatment, a prediction rule
28 was developed. Based on sex, age, localization of symptoms, tender- and swollen joint
29 count, C-reactive protein level, the severity of morning stiffness, and the presence of
30 rheumatoid factor and ACPA, a prediction score with a cutoff level between 6 and 8 revealed a positive predictive value of 84% and a negative predictive value of 91% (**chapter 6**).
31 Treatment with DMARDs and biologicals are used to modulate immune responses
32 in RA. Among other effects, it was shown that a TNF-alpha antagonist depleted activated
33 CD4+CD25+ T cells by binding to membrane-bound TNF-alpha, resulting in recovery of
34 the CD4+CD25+ regulatory T cells in patients with rheumatoid arthritis (**chapter 8**). The
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1 importance of the presence of functional regulatory T cells was emphasized in **chapter**
2 **9**. Whereas patients with rheumatoid arthritis reacted with a pro-inflammatory response
3 to the human cartilage glycoprotein 39, healthy individuals had an anti-inflammatory
4 response. The strength of the anti-inflammatory response was to such a degree that it
5 even suppressed other pro-inflammatory responses.

6 7 **Arthritis**

8 It is unknown whether every patient with undifferentiated arthritis has the potential
9 to develop rheumatoid arthritis. In this thesis, the assumption was made that some
10 patients with undifferentiated arthritis actually had rheumatoid arthritis, which was
11 not recognized as such yet. However, the clinical syndrome rheumatoid arthritis that
12 fulfills the ACR criteria for rheumatoid arthritis can be seen as an end stage rheumatoid
13 arthritis. Although the end product is destruction of the joint, the etiology may differ in
14 this heterogeneous group. This heterogeneity is emphasized by the different onset of
15 disease (**chapter 3**).

16 Recently it has become clear that patients with ACPAs represent a distinct disease sub-
17 set within the clinical syndrome rheumatoid arthritis (1;2). Having a shared epitope in
18 the HLA class II molecules predisposes to the development of ACPAs (3;4). Most patients
19 with ACPAs eventually show clinical characteristics of rheumatoid arthritis. However, it
20 takes up to 13 years to progress to the clinical syndrome of rheumatoid arthritis (5;6).
21 Somehow it requires an unidentified second hit to progress to clinical symptoms.

22 For patients without the presence of ACPA who have developed rheumatoid arthritis
23 it is unclear which factors play a role. So far, no obvious genetic factor has been found.
24 Like the danger model (7), one could propose that an undifferentiated arthritis for any
25 reason could predispose to persisting arthritis if the danger signal persists, or even
26 after a second hit may progress to a destructive rheumatoid arthritis. In that case, all
27 patients with undifferentiated arthritis have the potential to progress to a higher level,
28 rheumatoid arthritis, when the second event is provided. This is in contrast to the idea
29 that undifferentiated arthritis at presentation is actually already rheumatoid arthritis.
30 Because the biomarkers that make the difference have not been identified yet and the
31 percentage of patients with undifferentiated arthritis that progresses to rheumatoid
32 arthritis depends on the inclusion criteria of the study population, the prediction rule
33 (**chapter 6**) is based on clinical symptoms and laboratory findings.

34 35 **Monitoring undifferentiated arthritis**

36 There are different ways to monitor rheumatoid arthritis, but most of them have not
37 been validated in patients with undifferentiated arthritis. With the initiation of DMARDs
38 early in the disease process, the outcome variables may be less pronounced, possibly
39 requiring different methods for monitoring. Whereas in rheumatoid arthritis with a high

1 disease activity, the difference in improvement criteria and remission criteria is clear,
2 in undifferentiated arthritis the improvement criteria partially resemble the remission
3 criteria. Therefore, treatment goals should not be improvement, but remission in the
4 most strict way.

5 In **chapter 5** it was shown that the use of the original DAS, which was used in the
6 PROMPT study, was valid in patients with undifferentiated arthritis. The DAS28, however,
7 may lead to an underestimation of disease activity if only few joints are involved that are
8 not among the 28 counted, such as the feet. In undifferentiated arthritis, disease activity
9 is generally low, and by using extended joint counts including the feet an underestima-
10 tion of the number of joints involved may be prevented.

11 Although the DAS mirrors the disease activity in undifferentiated arthritis adequately,
12 the translation to clinical practice should be defined. A DAS of more than 2.4 in patients
13 with rheumatoid arthritis means an active disease state for most rheumatologists that
14 is high enough to intensify medication (8). A DAS of 1.6 is used as a cut off for remission
15 (9). However, accepting that a patient with initially undifferentiated arthritis who has a
16 DAS less than 1.6 with still arthritis in at least 1 joint would be considered to be in remis-
17 sion is contradictory, as 1 swollen joint was enough to enter the PROMPT study. In 1981
18 the ARA remission criteria for rheumatoid arthritis were published. For remission, five
19 or more of the following criteria must be fulfilled for at least two consecutive months:
20 duration of morning stiffness not exceeding 15 minutes, no fatigue, no joint pain (by his-
21 tory), no joint tenderness or pain on motion, no soft tissue swelling in joints or tendon
22 sheets, ESR less than 20 mm/h (male) or less than 30 mm/h (female) (10). Only 40% of
23 the PROMPT patients with a DAS less than 1.6 fulfilled the ARA remission criteria. This is
24 in contrast to patients with rheumatoid arthritis, where the DAS less than 1.6 correlates
25 well with the ARA criteria for remission (9). This emphasizes that the definitions used for
26 rheumatoid arthritis are not equally useful in undifferentiated arthritis. In the PROMPT
27 study, remission was therefore defined as no clinical symptoms of arthritis and further-
28 more no DMARD use. The role of imaging in the definition of remission is up for debate.
29 As ultrasound reveals more subclinical arthritis (11;12), one could argue that for remis-
30 sion and the treatment decision to stop DMARD therapy, even this subclinical arthritis
31 should be absent. The predictive value on the long term is unclear and the advantages
32 of continuing medication should be very carefully outweighed by the side-effects.

33 As erosive disease is very disabling, retarding bone erosions as quantified by radio-
34 graphic damage is a very important outcome measurement. Studies in clinical rheu-
35 matoid arthritis show that the best effect of DMARDs on radiographic progression is
36 when they are given in the first two years after rheumatoid arthritis is diagnosed (13-15).
37 However, the majority of the patients in the PROMPT study had no radiographic joint
38 progression in 18 months. If DMARD treatment is initiated in the window of opportunity
39 before damage occurs, radiographic damage cannot be accurately measured. The lack

1 of radiographic damage alone is inconclusive. Repair of erosions was not scored in the
2 PROMPT study, as it requires more patients with radiographic damage. Furthermore, it
3 was reported that repair of erosions was observed in patients with absence of clinical
4 synovitis for a longer period than the study time (16;17). As the radiographic damage
5 was clearly seen in ACPA positive patients in the PROMPT study, radiographic monitor-
6 ing should not be omitted, but for monitoring in early undifferentiated arthritis it is not
7 the best measurement.

9 **Treatment strategies in the window of opportunity**

10 Once clinical symptoms of arthritis do occur, starting a treatment is justified. For patients
11 who present with rheumatoid arthritis, DMARDs can be started immediately without
12 discussion. However, in patients with undifferentiated arthritis, from which 6-55% actu-
13 ally has rheumatoid arthritis (**chapter 2**), and thus 45-96% does not have rheumatoid
14 arthritis, it is very important to withhold a potential toxic treatment with DMARDs for
15 those who do not need it. To narrow down this very wide range a prediction rule was
16 designed (**chapter 6**). The ACR criteria for rheumatoid arthritis are not suitable for this
17 purpose, as these criteria were designed for describing the population of patients with
18 an established rheumatoid arthritis. Nor are the criteria for probable rheumatoid arthri-
19 tis, as all the patients in the PROMPT study fulfilled the criteria for probable rheumatoid
20 arthritis, and only 53% of the patients in the placebo group had rheumatoid arthritis after
21 18 months. In 2002 a prediction model to discriminate between self-limiting, persistent
22 non-erosive and persistent erosive arthritis was developed (18). In the present prediction
23 rule, the aim was not to predict persistent arthritis, but to predict rheumatoid arthritis
24 and the consequent justification for DMARD treatment. Depending on the cohort, no
25 adequate prediction could be made in 6 to 25% of the patients. These results have been
26 replicated in other cohorts (19-22). Although the prediction rule is a useful tool, there is
27 still some space for improvement by adding other factors, like genetic or environmental,
28 to simplify treatment decisions in the grey area in which no adequate prediction can be
29 made.

30 In an ideal situation, DMARD treatment will induce remission before permanent
31 damage has occurred. In the PROMPT study, the disease process could not be reversed,
32 although the intervention was done in the presumed window of opportunity (**chapter**
33 **4**). Maybe the duration of symptoms might have been too long for MTX to reverse the
34 process or MTX monotherapy is just not enough. It is tempting to extrapolate the data
35 from patients with early diagnosed rheumatoid arthritis to patients with undifferentiated
36 arthritis who actually have rheumatoid arthritis. The BeSt- en COBRA-study showed that
37 combination therapy is superior to monotherapy or step-up therapy (23;24). However,
38 there is little evidence-based information on the choice of treatment of undifferenti-
39 ated arthritis. Intra-articular corticosteroids in patients with oligoarthritis followed by

1 sulphasalazine in case of persistent or progressive disease resulted in more absence of
2 synovitis at 1 year when compared with NSAIDs followed by the same delayed interven-
3 tion if necessary (25). In patients with poor prognosis undifferentiated arthritis, which is
4 recurrence of synovitis after a single dose of corticosteroids with symptom duration of
5 less than 1 year, a randomized, placebo-controlled trial with infliximab provided mod-
6 est short-term relief, but did not prevent the development of rheumatoid arthritis (26).
7 Other double-blind randomized placebo-controlled studies in undifferentiated arthritis
8 comprise treatment with antibiotics in mixed populations of undifferentiated arthritis
9 and reactive arthritis and have had so far no effect on the disease course (27;28). Other
10 open label studies with DMARDs in patients with undifferentiated arthritis were per-
11 formed, but in mixed populations without a matched control group, and DMARDs were
12 also initiated further in the disease process (29;30). So far the best treatment strategy for
13 undifferentiated arthritis has not been found, though early intervention with DMARDs
14 shows beneficial effects with regard to symptom reduction and postponing the clinical
15 syndrome of rheumatoid arthritis.

16 ACPA-positive disease and ACPA-negative disease differ in histology, etiology and
17 genetic background (1;2). Although the number of patients in the PROMPT study who
18 were ACPA positive was small and it was a post-hoc analysis, the difference in the efficacy
19 of methotrexate was striking. Moreover, in ACPA-positive patients, patients with low and
20 intermediate levels of ACPA showed a more favorable response to MTX compared to pa-
21 tients with high levels of ACPA (**chapter 7**). If this corresponds to an earlier phase in the
22 development to the clinical syndrome rheumatoid arthritis, than a different treatment
23 strategy for patients with low and intermediate levels might be more appropriate. There
24 are no results of clinical trials with treatment strategies that distinguish between ACPA-
25 positive and -negative disease. This will provide an extra dimension towards tailor-made
26 treatment.

27 28 Immunomodulation

29 In autoimmune diseases the challenge is to stop the effect of the autoimmune
30 processes. To achieve this goal most treatment strategies aim at suppressing the im-
31 mune system by using agents that interfere with general processes like DNA synthesis,
32 cytokinesignalling or receptorsignalling. Irrespective of the origin of the autoimmune
33 disease or the affected organ these agents are successfully used. The counterpart of
34 these agents is the incidence of infections due to the lack in a pro-inflammatory immune
35 system. Biologicals, like anti-TNF-alpha, have had a major impact on the outcome of
36 rheumatoid arthritis. It was shown that the suppressive capability of CD4+ CD25+ T cells
37 was compromised in patients with active rheumatoid arthritis and was reversed after
38 anti-TNF-alpha therapy (31;32). The CD4+CD25+ membrane-bound TNF-alpha-negative
39 T cells showed suppressive capacities. Binding of adalimumab to membrane-bound

1 TNF-alpha resulted in depletion of activated CD4+CD25+ T cells and restoration of the
2 suppressive capacity of the remaining functional CD4+CD25+ membrane-bound TNF-
3 alpha-negative T cells. The presence of membrane-bound TNF-alpha on CD4+CD25+ T
4 cells was correlated to disease activity in rheumatoid arthritis patients (**chapter 8**). This
5 emphasizes the imbalance between the pro-inflammatory response and the regulatory
6 T cell response in patients with rheumatoid arthritis. This imbalance was also found in
7 the immunologic response to HC-gp39. T cells from rheumatoid arthritis patients pro-
8 duced anti-inflammatory cytokines like IFN-gamma in response to stimulation with HC-
9 gp39, whereas T cells from healthy individuals reacted with producing IL-10. T cell lines
10 against HC-gp39 from healthy individuals were able to suppress a pro-inflammatory
11 response from a T cell line against a peptide from mycobacterium, suggesting that the
12 response with IL-10 production was a regulatory response. Furthermore, the T cell lines
13 against HC-gp39 from healthy individuals were CD4+ and expressed high levels of CD25
14 (**chapter 9**).

15 Understanding the mechanisms in autoimmunity will provide tools in developing
16 more specific treatment strategies. Autoimmune diseases occur in up to 3-5% of the gen-
17 eral population. Both genetic and environmental factors contribute to the susceptibility
18 to autoimmunity (33). In this thesis, T cell mediated immunoregulation is addressed. It
19 is postulated that the immune system distinguishes between the absence and presence
20 of danger signals in combination with either self or non-self peptides. In the presence
21 of a danger signal, like pathogen-associated molecular patterns (PAMPs) binding to pat-
22 tern recognition receptors and non-programmed cell death, the immune response is
23 directed towards a pro-inflammatory response. In combination with a self peptide this
24 will result in autoimmunity (7;34). The described imbalance between pro-inflammatory
25 and regulatory immune responses implies that skewing the immune response from pro-
26 inflammatory to anti-inflammatory or restoring the suppressor function at specific sites
27 might invert the autoimmune process. As DMARDs and biologicals are broad spectrum
28 agents that potentially react on every inflammation in the body, the ultimate challenge
29 in autoimmunity will be to interfere within the immune processes more specifically. HC-
30 gp39 might be a candidate auto antigen to target for redirecting the immune response
31 as it is one of the major constituents of human cartilage, the mRNA and protein are
32 upregulated in synovium of rheumatoid arthritis patients and T cell-epitopes from HC
33 gp-39 are presented by antigen presenting cells in synovium of rheumatoid arthritis
34 patients (35-39). In a mouse model, intranasal treatment with HC-gp39 suppressed
35 HC-gp39-induced and collagen-induced arthritis (40;41). However, tolerance induction
36 in rodents is possible via specialised superficial cervical and internal jugular draining
37 lymphnodes of the nose. Other lymphnodes do not have this capacity (42). It is difficult to
38 perform such controlled experiments with respect to dosing and stage of the disease in
39

1 humans. Here, it was shown that the response to HC-gp39 differs between healthy donors
2 and rheumatoid arthritis patients.

3 In an attempt to influence the immunological response to HC gp-39 in order to influ-
4 ence disease outcome, a phase I trial was performed. Briefly, rheumatoid arthritis pa-
5 tients with moderate disease activity were treated with once weekly 25 µg, 125 µg, 625
6 µg or 3125 µg HC gp-39 intranasally during 4 weeks, and followed for another 8 weeks.
7 In every treatment group, eight patients received HC gp-39 and two patients received
8 placebo. No severe adverse events were reported, however, a clinical significant effect
9 on the disease activity of the rheumatoid arthritis patients was not seen either (43). To
10 determine the underlying immunological reaction, peripheral blood mononuclear cells
11 (PBMCs) were isolated and cryopreserved from patients with RA who participated in
12 the phase I clinical trial. Blood samples were taken before entering the trial, after ap-
13 proximately 4 weeks of treatment and after an 8-week HC gp-39 free interval being
14 approximately 12 weeks after entering the trial. The HC gp-39 response was not tested
15 before entering the trial. PBMCs of only 23 patients were available for analysis. Differ-
16 ence at the level of cytokine production as measured by cytometric bead arrays and in
17 enzyme linked immuno sorbent culture did not distinguish the placebo group from the
18 four treatment groups (H. van Dongen, unpublished results). The obtained results were
19 not eligible for publication for several reasons. Blood samples from only 23 out of 40
20 patients were available for the analysis. As the remaining initial groups were too small
21 for correct statistical testing, a proof of principle would be the only possibility. After
22 selecting the patients based on clinical outcome, the groups were also too small, and
23 the results differed per patient. Induction to tolerance against HC-gp39 in humans has
24 not been performed yet. The fact that HC-gp39 is probably not the antigen that drives
25 the expansion of T cells to an autoimmune process should be irrelevant as long as the
26 response to HC-gp39 is strong enough to suppress the other immune responses in the
27 joint. This might even be an advantage, as a reduced frequency of nickel hypersensitiv-
28 ity was reported after oral nickel contact at an early age by oral braces. The reduced
29 frequency was only seen if the oral contact was prior to sensitization to nickel by i.e.
30 earpearing (44). Perhaps HC-gp39 should be administered to patients in rheumatoid
31 arthritis who do not show a pro-inflammatory response to HC-gp39 (yet), to reinforce
32 the existing anti-inflammatory response and will hopefully suppress other inflammatory
33 responses as HC-gp39 is up regulated in the inflamed synovium. In mice, collagen type II
34 induced arthritis is also best prevented when the feeding of collagen type II starts before
35 the induction of arthritis. Studies concerning oral tolerance induction in rheumatoid
36 arthritis patients have been performed, but clinical efficacy was poor and functional
37 evidence of tolerance was not convincing, as cytokine profiles and markers like FoxP3
38 and CD25 are not specific enough, or even absent (45;46). Therefore, no conclusions
39 could be drawn.

1 Future perspectives

2 Undifferentiated arthritis remains a challenging state of disease. The outcome varies
3 from self limiting to severe destructive arthritis. As the evidence for early treatment of
4 rheumatoid arthritis is accumulating, the urge for selecting patients early in the disease
5 course increases. As genetic susceptibility is already present at birth and ACPA can be
6 detected up to 14 years before clinical symptoms of arthritis occur, there will be no
7 support for starting a treatment in the preclinical phase (5;6). The choice of treatment
8 should be crystallized in clinical trials in patients with undifferentiated arthritis. When
9 patients with rheumatoid arthritis are distinguishable in a population of patients with
10 undifferentiated arthritis, and the immunologic reaction is not widespread, perhaps oral
11 tolerance induction will be possible. Many candidate auto antigens have been investi-
12 gated, but most of them do not make it from bench to bedside. For HC-gp39 the curtains
13 haven't closed yet. More translational research regarding this aspect is necessary.

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CHAPTER 11

Nederlandse samenvatting

1 Nederlandse samenvatting

2
3 Het onderzoek dat beschreven wordt in dit proefschrift bestaat uit twee delen: **Deel I**
4 gaat over ongedifferentieerde artritis, de progressie daarvan naar reumatoïde artritis,
5 het resultaat van agressieve behandeling in een vroeg stadium van reumatoïde artritis
6 en hoe te voorspellen welke patiënten daarvoor in aanmerking komen. In **deel II** worden
7 enige immunologische aspecten van reumatoïde artritis beschreven.

10 Deel I: Ongedifferentieerde artritis

12 Artritis

13 Een gewrichtsontsteking of artritis wordt gekenmerkt door warmte, pijn en zwelling in
14 een of meerdere gewrichten. Reumatoïde artritis is de meest voorkomende chronisch
15 inflammatoire gewrichtsaandoening en komt bij 1% van de bevolking voor, waarbij
16 meer vrouwen dan mannen zijn aangedaan. Het is belangrijk om reumatoïde artritis
17 in een vroeg stadium te herkennen, omdat deze ziekte onbehandeld uiteindelijk leidt
18 tot destructie van het gewricht. Door het starten van behandeling zodra de diagnose
19 reumatoïde artritis duidelijk is, kan deze schade voorkomen worden. Om reumatoïde
20 artritis in een vroeg stadium te onderzoeken, zijn zogenaamde 'early arthritis clinics'
21 opgezet. Vanaf de jaren 50 zijn er criteria ontworpen om de verschillende vormen van
22 artritis in te delen. Hierbij worden o.a. het voorkomen van ochtendstijfheid, het soort en
23 aantal aangedane gewrichten, de aanwezigheid van antilichamen zoals reumafactor, en
24 al bestaande gewrichtsschade beoordeeld (zie ook tabel 1, **hoofdstuk 1**). Als een artritis
25 niet valt te categorizeren in een van de ziekteëntiteiten die is gedefinieerd door de Ame-
26 rican College of Rheumatology (ACR), betreft het een ongedifferentieerde artritis. Uit de
27 Leidse 'early arthritis clinic' bleek dat 37% van de patiënten die de polikliniek bezocht met
28 een artritis een ongedifferentieerde artritis had. Na een jaar had 30% van deze patiënten
29 geen artritis meer. 28% bleek echter een reumatoïde artritis te hebben. In **hoofdstuk 2**
30 is onderzocht hoe vaak patiënten met ongedifferentieerde artritis in verschillende 'early
31 arthritis clinics' wereldwijd binnen 1 jaar reumatoïde artritis ontwikkelden. Afhankelijk
32 van het soort en het aantal gewrichten dat is aangedaan bleek 6-55% van de patiënten
33 met een ongedifferentieerde artritis binnen 1 jaar reumatoïde artritis te hebben. Indien
34 werd vereist dat patiënten bij een eerste beoordeling door een reumatoloog een artritis
35 hadden en dat voor de diagnose reumatoïde artritis moest worden voldaan aan de clas-
36 sificatie criteria zoals opgesteld door de ACR, bleek 17-32% van de patiënten binnen 1
37 jaar reumatoïde artritis te hebben.

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1 Beoordelen en vervolgen van artritis

2 Reumatoïde artritis wordt gekenmerkt door toenemende destructie van gewrichten, die
3 uiteindelijk tot invaliditeit leiden. Er zijn verschillende methoden om de ernst van een
4 artritis en de ziekte activiteit te beoordelen en te vervolgen. Deze methoden kunnen
5 gebruikt worden om de effectiviteit van therapie te beoordelen, of als uitgangspunt om
6 wel of geen medicatie te starten.

7 De 'disease activity score', kortweg DAS, is een formule die is gebaseerd op een aantal
8 pijnlijke gewrichten, een aantal gezwollen gewrichten, de hoogte van een onstekings-
9 parameter in het bloed (bezinking), en de beoordeling van algemeen welbevinden op
10 een schaal van een tot tien door de patiënt. De DAS was gebaseerd op ziekteactiviteit in
11 patiënten met reumatoïde artritis. In **hoofdstuk 5** blijkt dat de DAS score ook toepasbaar
12 is in patiënten met een ongedifferentieerde artritis. In patiënten met reumatoïde artritis
13 is een DAS van 2,4 reden om medicatie op te hogen, en een DAS van 1,6 wordt gebruikt
14 als afkapwaarde voor remissie. Echter in patiënten met een ongedifferentieerde artritis,
15 die per definitie minder pijnlijke en gezwollen gewrichten hebben, kan de DAS 1,6 zijn
16 terwijl er artritis is. Met de huidige behandelstrategieën en het toenemend bewijs voor
17 het starten van agressieve therapieën vroeg in het ziekte proces zal de definitie van
18 remissie, al dan niet op basis van de DAS, voor patiënten met ongedifferentieerde artritis
19 aangepast moeten worden.

20 Beeldvorming van het aangedane gewricht speelt ook een rol in de beoordeling van
21 de ernst van de reumatoïde artritis. De Sharp-van der Heijde gemodificeerde scorings-
22 methode is een manier om röntgenfoto's van handen en voeten te beoordelen op het
23 optreden van gewrichtsspleetversmalling en erosies (zie figuur 1, **hoofdstuk 1**). Hoe
24 hoger de score, des te meer schade aan het gewricht. Deze manier van beoordelen is
25 ook gebruikt in de onderzoeken beschreven in dit proefschrift.

26 Andere manieren om de ernst van de ziekte te beoordelen zijn meer subjectieve
27 methoden met vragenlijsten naar algemeen functioneren, zoals de 'health assessment
28 questionnaire' (HAQ), de 'arthritis impact measurement scales' (AIMS), en de 'rheumatoid
29 arthritis disease activity index' (RADAI).

30

31 Beargumentatie voor behandeling vroeg in het ziekteproces

32 De afgelopen twintig jaar is de behandeling van reumatoïde artritis sterk veranderd.
33 Voor 1994 werd een patiënt met nieuw gediagnostiseerde reumatoïde artritis behandeld
34 volgens de pyramide strategie. Er werd dan begonnen met een ontstekingsremmer, een
35 zogenaamde 'non-steroid anti-inflammatory drug' (NSAID) en pas bij ernstige ziekte
36 werd gestart met een 'disease-modifying anti-rheumatic drug' (DMARD). Met een NSAID
37 werden wel de klachten verminderd, maar van NSAIDs wordt niet gedacht dat ze het
38 onderliggende ziekteproces beïnvloeden zoals bij DMARDs. Later bleek dat patiënten
39 die bij de diagnose reumatoïde artritis gelijk startten met een DMARD minder schade

1 aan de gewrichten op röntgenfoto's van handen en voeten en een lagere ziekteakti-
2 viteit hadden dan patiënten behandeld volgens de pyramide strategie. Het grootste
3 effect werd daarbij in het eerste jaar van behandeling gezien. Na 10 jaar was dit effect
4 nog steeds zichtbaar in het aantal operaties dat nodig was als gevolg van opgelopen
5 gewrichtsschade. Het lijkt er dus op dat hoe eerder er in het ziekteproces ingegrepen
6 wordt, des te beter de uitkomst op lange termijn is. In **hoofdstuk 3** is onderzocht of
7 patiënten die zich presenteren met een ongedifferentieerde artritis en binnen een jaar
8 reumatoïde artritis ontwikkelen een milder ziektebeeld hebben dan patiënten die zich
9 presenteren met een duidelijke reumatoïde artritis. Het blijkt dat in een tijdsbestek
10 van vier jaar de patiënten die in het begin een ongedifferentieerde artritis hadden net
11 zo veel gewrichtsbeschadiging en ziekteactiviteit hadden ontwikkeld als diegenen
12 die in het begin een duidelijke reumatoïde artritis hadden. Wanneer het grootste ef-
13 fect van behandeling in het eerste jaar wordt gezien, zou het starten van DMARDs al
14 in het stadium van ongedifferentieerde artritis gerechtvaardigd zijn. Deze aanname
15 is onderzocht in de *PRObable RA: Methotrexate versus Placebo Treatment (PROMPT)*
16 studie (**hoofdstuk 4**). In de PROMPT studie zijn patiënten met een ongedifferentieerde
17 artritis behandeld met methotrexaat of placebo. In de methotrexaat groep bleek dat
18 progressie van het ziektebeeld naar een duidelijke reumatoïde artritis uitgesteld was en
19 dat er minder schade op röntgenfoto's te zien was dan in de placebo groep. In patiënten
20 met antistoffen tegen gecitrullineerde peptiden (ACPAs) waren de resultaten nog meer
21 uitgesproken (**hoofdstuk 4 and 7**).

22 Recent is duidelijk geworden dat patiënten die ACPAs hebben een aparte groep
23 vormen binnen de groep patiënten met een reumatoïde artritis. Het hebben van een
24 bepaalde gezamenlijke aminozuursequentie, de zogenaamde 'shared epitope', in human
25 leucocyte antigen (HLA) klasse II moleculen is een aanleg voor het ontwikkelen van
26 ACPAs. De meeste patiënten met ACPAs ontwikkelen uiteindelijk reumatoïde artritis.
27 Het kan echter wel 13 jaar duren voor dit plaats vindt. Op een of andere manier is er een
28 onbekende tweede factor nodig om klinische verschijnselen te ontwikkelen (zie figuur
29 2, **hoofdstuk 1**). Voor patiënten die geen ACPAs hebben, maar wel reumatoïde artritis, is
30 het nog onduidelijk welke andere factoren een rol spelen.

31 Binnen de PROMPT studie was de incidentie van reumatoïde artritis lager in patiënten
32 die lage of intermediaire ACPA titers hadden en behandeld werden met methotrexaat
33 dan in patiënten met een hoge antistoftiter. In een groep patiënten die nooit eerder was
34 behandeld met een 'disease-modifying anti-rheumatic drug' (DMARD) en gediagnos-
35 tiseerd waren met ongedifferentieerde artritis of een net ontstane reumatoïde artritis
36 bleek dat lage en intermediaire ACPA titers geassocieerd waren met een betere reactie
37 op methotrexaat behandeling dan patiënten met hoge antistoftiters (**hoofdstuk 7**).

38 Om te bepalen welke patiënt met een ongedifferentieerde artritis eigenlijk reumato-
39 ide artritis heeft, of waarschijnlijk zal ontwikkelen, en dus wie baat zou hebben bij een

1 vroege behandeling met een DMARD, is een voorspelregel ontwikkeld. Op basis van
2 geslacht, leeftijd, plaats van de klachten, het aantal pijnlijke en gezwollen gewrichten,
3 de hoogte van het C-reactieve proteïne, de mate van ochtendstijfheid, en de aanwe-
4 zigheid van reumafactor en ACPA leverde een voorspellende score met afkapwaarden
5 tussen de 6 en 8 punten een positief voorspellende waarde van 84% en een negatief
6 voorspellende waarde van 91% op (**hoofdstuk 6**).

9 **Deel II: Reumatoïde artritis**

10

11 Reumatoïde artritis is een zogenaamde auto-immuunziekte waarbij het immuunsysteem
12 zorgt voor een ontstekingsproces in de gewrichten met als eind resultaat destructie van
13 het gewricht. De gedachte is dat door een combinatie van genetische factoren en uitlok-
14 kende omgevingsfactoren schadelijke T cellen worden geactiveerd die onder andere B
15 cellen kunnen stimuleren en daarmee de antistofproductie op gang brengen (zie figuur
16 2, **hoofdstuk 1**). Verschillende onderdelen van deze cascade van gebeurtenissen zijn
17 onderzocht.

18 De invloed van genetisch factoren blijkt uit een drie tot vier keer zo vaak voorkomen
19 van reumatoïde artritis in een-eïge in vergelijking tot twee-eïge tweelingen. De totale
20 genetische bijdrage wordt geschat op 50 tot 60 %. De meest bekende risicofactor tot nu
21 toe is de 'shared epitope' in het HLA klasse II gen.

22

23 **T cellen**

24 De betrokkenheid van HLA klasse II moleculen en de aanwezigheid van T cellen gericht
25 tegen eiwitten afkomstig uit het gewricht suggereren een rol voor CD4+ T cellen. Nadat
26 de T cellen zijn ontstaan uit voorlopercellen in het beenmerg, verhuizen ze naar de thy-
27 mus om daar het onderscheid te leren maken tussen lichaamsvreemde en lichaamsei-
28 gen eiwitten, gepresenteerd in eigen HLA moleculen. Allereerst vindt positieve selectie
29 plaats van die T cellen die peptiden herkennen in de context van eigen HLA moleculen.
30 Daarna vindt een negatieve selectie procedure plaats waarbij T cellen die reageren op
31 lichaamseigen eiwitten geëlimineerd worden. Grofweg bestaan er twee typen T cellen.
32 Cytotoxische T cellen die gekenmerkt worden door CD8 expressie en die antigenen
33 herkennen in de context van HLA klasse I moleculen. Deze moleculen zitten op vrijwel
34 alle cellen met een celkern en presenteren intracellulaire eiwitten. Als de CD8+ T cel
35 geactiveerd wordt, doodt de T cel de antigeen presenterende cel door cellysis. Tijdens
36 aktivatie van CD8+ T cellen komen pro-inflammatoire cytokinen als interferon-gamma,
37 tumor necrosis factor- alfa en interleukine-2 vrij. T helper cellen brengen CD4 op hun
38 celmembraan tot expressie en herkennen antigenen in de context van HLA klasse II.
39 Alleen antigeen presenterende cellen als dendritische cellen, monocytten, macrofagen

1 en B cellen kunnen extracellulaire eiwitten opnemen, bewerken en presenteren in HLA
2 klasse II moleculen. Wanneer de T helper cel geactiveerd wordt, kan deze B cellen
3 activeren die vervolgens van bepaalde antistoffen gaan produceren. Deze processen
4 worden gekenmerkt door het vrij komen van interleukine-4 en interleukine-10.

5 Bovenstaande processen verklaren echter niet waarom regulatoire of suppressor
6 T cellen bestaan en waarom er autoimmuunziekten kunnen ontstaan. Er bestaan ver-
7 schillende hypothesen over de origine van autoimmuniteit, variërend van genetische
8 defecten die het selectie proces in de thymus beïnvloeden tot 'danger models' waarin
9 beschadigde weefsels een waarschuwingssignaal afgeven, waarbij dit waarschuwings-
10 signaal fungeert als co-stimulerende factor voor activatie van een autoimmunproces.
11 Het bestaan van regulatoire T cellen is functioneel voor het eerst aangetoond in
12 naakte muizen waarbij infusie van CD25+CD4+ T cellen na infusie van CD25-CD4+ T
13 cellen voorkwam dat er een autoimmuunziekte ontstond. CD25 is de alfa keten van de
14 interleukine-2 receptor. Bij analyse met behulp van flowcytometrie komt CD25 hoog tot
15 expressie op geactiveerde T cellen vroeg in de activatiefase, terwijl er een intermediair
16 expressie patroon op regulatoire T cellen wordt beschreven. Ander celmarkers, zoals
17 'cytotoxic T lymphocyte antigen-4' (CTLA-4), 'glucocorticoid induced TNF receptor (GITR)
18 en FoxP3 worden ook wel gebruikt om regulatoire T cellen te beschrijven, maar deze
19 markers zijn ook niet specifiek. De regulatoire T cel reactie wordt gekenmerkt door
20 het vrijkomen van anti-inflammatoire cytokinen, zoals interleukine-10 en tumor groei
21 factor-beta. T cel gemedieerde immunoregulatie speelt waarschijnlijk wel een rol in de
22 tolerantie van lichaamseigen eiwitten, maar de exacte rol van regulatoire T cellen blijft
23 onduidelijk, vooral in de mens.

24 De uitdaging in autoimmuunziekten is het beëindigen van het autoimmunproces.
25 Behandeling met DMARDs en zogenaamde 'biologicals', zoals TNF-alfa antagonisten,
26 worden gebruikt om de immunologische processen in onder andere patiënten met
27 reumatoïde artritis te beïnvloeden. Het bleek dat de suppressieve capaciteit van
28 CD4+CD25+ T cellen in patiënten met reumatoïde artritis was verminderd, en weer
29 hersteld na anti-TNF-alfa therapie. In **hoofdstuk 8** bleek dat de aanwezigheid van
30 membraangebonden TNF-alfa op CD4+ CD25+ cellen samenhang met ziekteactiviteit in
31 patiënten met reumatoïde artritis. Binding van een gehumaniseerde TNF-alfa antago-
32 nist, adalimumab, aan membraangebonden TNF-alfa resulteerde in het uitschakelen van
33 geactiveerde CD4+CD25+ T cellen, waardoor de functie van CD4+CD25+ regulatoire T
34 cellen hersteld werd. Dit benadrukt de onevenwichtigheid tussen de pro-inflammatoire
35 reactie en de regulatoire T cel reactie in patiënten met reumatoïde artritis.

36 Deze onevenwichtigheid wordt ook gevonden in de immunologische reactie op het
37 humane kraakbeen glycoproteïne-39 (HC gp-39). In **hoofdstuk 9** blijkt dat patiënten met
38 reumatoïde artritis een pro-inflammatoire reactie hadden op HC gp-39, terwijl gezonde
39 personen een anti-inflammatoire reactie hadden. De sterkte van deze anti-inflammatoire

1 reactie was zo groot dat deze andere pro-inflammatoire reacties kon onderdrukken.
2 Bovendien maakt HC gp-39 een groot deel uit van het humane kraakbeen, het mRNA en
3 het eiwit zelf komen verhoogd tot expressie in het synovium van patiënten met reuma-
4 toïde artritis en delen van HC gp-39 worden door antigeen presenterende cellen in het
5 synovium van patiënten met reumatoïde artritis gepresenteerd aan T cellen. HC gp-39
6 zou daarmee een potentiëel middel kunnen zijn om het autoimmunproces in reuma-
7 toïde artritis te beïnvloeden. In een muismodel bleek dat intranasaal toedienen van HC
8 gp-39 het ontstaan van artritis kon onderdrukken. Naar analogie hiervan werd een fase
9 I studie in patiënten met reumatoïde artritis opgezet, waarbij HC gp-39 gedurende 4
10 weken wekelijks werd toegediend. Daarna werden de patiënten nog 8 weken beoor-
11 deeld op ziekte activiteit. Helaas bleek er klinisch geen duidelijk effect waarneembaar.
12 Om onderliggende immunologische reacties te analyseren, zijn tijdens deze fase I studie
13 bloedmonsters afgenomen. In de **Summary and discussion** worden de resultaten van
14 deze analyse besproken. Helaas was er voor deelname aan de fase I studie niet getest
15 op de aanwezigheid van een pro-inflammatoire reactie op HC gp-39, waardoor de groep
16 patiënten zeer heterogeen bleek. Daar niet van alle patiënten een monster beschikbaar
17 was, en per type HC-gp-39 reactie te weinig patiënten per groep waren, was het niet
18 mogelijk om hier een goede conclusie uit te trekken. Daarbij komt nog dat knaagdieren
19 mogelijk een ander werkingsmechanisme van tolerantie inductie hebben dan mensen,
20 er geen duidelijk klinisch effect waarneembaar was in de patiënten in de fase I studie, en
21 het niet duidelijk is of in de fase I studie de best mogelijke doseringen zijn gebruikt. Der-
22 halve zijn de resultaten van dit laatste project niet gepubliceerd. Dat tolerantie inductie
23 mogelijk is bij mensen blijkt uit het feit dat bij mensen die op jonge leeftijd een beugel
24 of piercing gehad hebben, op latere leeftijd minder vaak nikkel allergieën voorkomen.

25 26 **Toekomstperspectieven**

27 Ongedifferentieerde artritis blijft klinisch een enorme uitdaging. Er is steeds meer be-
28 wijs dat vroeg starten van een behandeling in patiënten met reumatoïde artritis zeer
29 gunstige effecten heeft op het latere beloop van de ziekte. Des te belangrijker is het
30 om de reumatoïde artritis patiënt al in een vroeg stadium te herkennen. De keuze van
31 therapie lijkt voorsnóg verschillend te zijn in verschillende stadia van de ziekte. Waar
32 het beste mee gestart kan worden in de fase van een ongedifferentieerde artritis zal nog
33 verder uitgezocht moeten worden. Tevens lijkt de aanwezigheid van ACPAs al in een
34 vroeg stadium van belang. Het is nog niet uitgesloten dat orale tolerantie inductie een
35 mogelijkheid is, en ook voor HC gp-39 zouden er theoretisch nog mogelijkheden zijn.

Curriculum vitae

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3 Henrike werd op 16 december 1975 geboren in Dordrecht. In 1994 werd het V.W.O.-
4 diploma behaald aan Dordtswijk, locatie Noordendijk, te Dordrecht, waarna zij startte
5 met de studie Biomedische Wetenschappen aan de Universiteit Leiden. In 1996 startte
6 zij tevens met de studie Geneeskunde aan de Universiteit Leiden. Zij heeft een weten-
7 schapsstage gelopen bij de afdeling Reumatologie (begeleiding: prof. dr. T.W.J. Huizinga,
8 prof. dr. C.L. Verwey, dr. E.L. Kaijzel) getiteld 'Associative and functional aspects of four in-
9 terleukin-1 gene polymorphisms in rheumatoid arthritis', resulterend in een publicatie. In
10 Dormaa Presby Hospital, Dormaa Ahenkro, Ghana, heeft zij een klinische stage gelopen
11 (begeleiding: drs. C. Brumsen, A. Owusu Ansah). Haar afstudeerstage vond plaats bij de
12 Afdeling Immunologische en Infectieziekten, TNO Preventie en Gezondheid te Leiden,
13 getiteld 'Alpha B-crystallin as key autoantigen in a mouse model for multiple sclerosis' en
14 eveneens resulterend in een publicatie (begeleiding: dr. J.M. van Noort, dr. R. Verbeek).
15 De doctoraalexamens Biomedische Wetenschappen en Geneeskunde werden in 2001
16 behaald, waarna het artsexamen in 2003 volgde.

17 In april 2003 ving zij aan met het in dit proefschrift beschreven promotieonderzoek
18 bij de afdeling Reumatologie onder begeleiding van prof. dr. T.W.J. Huizinga en prof. dr.
19 R.E.M. Toes, waarvoor zij een persoonsgebonden ZonMw AGIKO-stipendium ontving.
20 Voor een deel van dit onderzoek werd zij genomineerd voor de Abott Young Investigator
21 Award in 2005 en ontving zij een Euler Abbott Abstract Award in 2006. Vanaf 2003 volgt
22 zij de postdoctorale opleiding tot immunoloog van de Stichting opleiding tot Medisch-
23 Biologisch Wetenschappelijk Onderzoeker (SMBWO).

24 Van mei 2006 tot en met december 2008 is zij gestart als arts in opleiding tot specialist
25 (AIOS) in de Reumatologie bij de afdeling Interne geneeskunde in het Medisch Centrum
26 Haaglanden te Den Haag (opleider mw. dr. P.H.L.M. Geelhoed-Duijvestijn). Van januari
27 2009 tot en met april 2010 heeft zij haar vooropleiding Interne geneeskunde vervolgd
28 in het Leids Universitair Medisch Centrum (LUMC) op de afdeling Interne Geneeskunde
29 (opleider prof. dr. J.A. Romijn). Vanaf 1 mei 2010 is zij werkzaam als AIOS Reumatologie
30 op de afdeling Reumatologie in het LUMC (opleider prof. dr. T.W.J. Huizinga).

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