Review

Can rheumatoid arthritis responsiveness to methotrexate and biologics be predicted?

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This review briefly recapitulates the existing markers predictive of RA responsiveness to treatment, focusing on MTX alone or combined with a biologic. In addition to the demographic and clinical factors, an update is provided of the predictive biomarkers identified by large-scale gene and protein analyses that generated new insights into the ability of high-throughput analysis of biological systems to select new potential indicators. Among the large-scale analysis tools now available, pharmacogenetics and pharmacogenomics (including transcriptomic and proteomic approaches) have been shown to provide such new putative biomarkers of therapeutic responses.

KEY WORDS: Rheumatoid arthritis, Methotrexate, Biologics, Biomarkers predictive of response, Pharmacogenetics, Pharmacogenomics.

Introduction

RA, the most common inflammatory autoimmune disease affecting joints, is characterized by synovial pannus formation that leads to cartilage destruction and bone erosion. Among DMARDs, MTX is the most commonly used, and is generally considered the anchor drug for biologics [1, 2]. Although MTX has proven efficacy when taken alone, it fails to control disease activity and structural damage in numerous patients. Over the past decade, elucidation of RA pathogenic mechanisms has led to the discovery of potential targets for its treatment.

These new targeted therapies are mainly directed against cytokines or cellular proteins. The former modulate TNF-α or IL-1, which play pivotal roles in RA pathogenesis, by neutralizing their actions via human or chimeric mAbs (i.e. adalimumab and infliximab), a soluble receptor (etanercept) or by using a recombinant-antagonist form of the cytokine receptor (anakinra). The latter exert their actions via mAbs directed against a cellular marker (rituximab) or via a recombinant immunoglobulin (Ig) targeting a co-stimulatory molecule (for example, abatacept). Biotherapies have improved the control of severe RA in the majority of patients and provide a therapeutic alternative for patients whose disease is inadequately controlled by MTX [3, 4]. However, in most cases, these agents are prescribed in combination with MTX.

Despite the breakthroughs made in the management of RA, a persistent subset of patients (30–40%) does not respond or poorly responds to these biologies, which means a prejudicial loss of time for the patient [5, 6]. Moreover, considering the potential severe side effects of these molecules and their high cost, the identification of patients who will most likely respond could contribute to optimizing their prescription, i.e. shorten the time to the onset of effective treatment, favour less empirical prescription and improve the cost/benefit ratio of these agents. Consequently, identifying predictors of the therapeutic response would be a major advance in the treatment of RA.

In 1999, Bridges [7] reported that no markers predictive of therapeutic response were known. Herein, we take stock of our current knowledge concerning predictive markers of RA responses to MTX and/or biologics. Demographic, inflammation and immunological factors of RA or new biomolecular tools, such as pharmacogenetics and pharmacogenomics, are among the approaches available to identify putative candidate biomarkers to predict RA response to MTX alone or combined with biotherapy.

Tools for the identification of markers predictive of RA therapeutic responses

The RA therapeutic response is assessed with clinical [disease activity score 28 (DAS28) or ACR criteria], structural (Sharp or Larsen scores) and/or functional evaluation standards (HAQ score). Comparing these assessments before and after treatment provides an estimation of treatment efficacy.

Among the putative predictive markers of the therapeutic response, demographic characteristics, like sex, age or disease duration and clinical characteristics might prove to be highly relevant. Several markers routinely used for RA diagnosis, e.g. CRP, COMP and RF levels and, more recently, autoantibodies might also help identify predictive indicators. This review details as to when these markers are able to identify responders to MTX alone or combined with a biotherapy (biologic–MTX). Today, other experimental approaches, such as large-scale gene and protein analyses have provided new perspectives for high-throughput analysis of biological systems to discover potential predictive markers. As a consequence, new fields of study have emerged, for example, pharmacogenetics and pharmacogenomics, which covers transcriptomic and proteomic studies.

Pharmacogenetics

The combination of a large number of polymorphisms identified with high-throughput genotyping methods opened unprecedented ways to ascertain factors potentially linked to disease activity and drug metabolism. Because some patients respond better to one TNF-α blocker than the others [8], their genetic backgrounds might influence their therapeutic responses. New putative predictive markers could be found by pharmacogenetics, which is the study of inherited predisposition and metabolic differences that
influence interindividual variability of responses to drugs [9]. More specifically, pharmacogenetics studies the genetic polymorphisms that correspond to sequence variations in a gene, like single nucleotide polymorphisms (SNPs). SNPs, which arise at a frequency of $\geq 1\%$, are single base changes that can occur at any site in the DNA molecule, in the 3’-regulatory sequence (promoter), the coding region or the untranslated 3’-region after the coding sequence. SNPs may have marked impact on a patient’s response to a particular agent [10]. Pharmacogenetics has investigated genetic variants influencing the response to immunotherapies, mainly by examining cytokine-gene polymorphisms (that could modulate the response to a drug) or promoter polymorphisms (that could intervene in the drug’s metabolic pathway), MHC gene or Fc receptor polymorphisms.

According to Bridges, the therapeutic response is not dependent on a unique gene but on multiple genes interacting which one another [11].

**Pharmacogenomics**

Transcriptomic analyses. One way to examine multiple gene products is the transcriptomic approach (microarrays), which enables the monitoring of the expression levels of several thousand genes simultaneously, by measuring their mRNA contents, at a given time and in a well-defined disease or healthy state. Microarrays have already been used to investigate various aspects of RA and their potential applications in this field were reviewed several years ago [12–17]. Thus, gene chips seem to be a good tool to identify biomarkers predictive of response among selected transcripts without any a priori and might be able to choose a drug at the individual patient level.

Proteomic analyses. While transcriptomics enables the assessment of the amounts of tens of thousands of gene transcripts, proteomics is the characterization and quantification of up to a few thousand proteins in tissues and body fluids. It can be used to classify individual patients and subsets of patients based on their ‘autoantibody fingerprints’, to discover and characterize candidate autoantigens, and to tailor antigen-specific therapy [18–20]. This technique is rapid and sensitive, and provides a profile of low-molecular weight peptides and proteins within a complex material, like serum. Hence, proteomic analysis could help select targeted proteins able to predict response to immunotherapies.

Transcriptomic and proteomic analyses are complementary approaches that provide information concerning potential targets at a given time but, it must be kept in mind, that transcript levels, for example, determined at that time do not necessarily reflect production of the corresponding protein.

**Markers of the response to MTX**

Demographic and clinical factors

Few authors have explored whether demographic factors, e.g. age or sex, could have an influence on the variation of RA responses to MTX. Bologna et al. [21] reported a consistent lack of an age effect on the RA response to MTX. So far, a relationship between disease duration and response has not yet been clearly established and remains controversial. However, a poorer response rate was associated with female sex, assessed with ACR20 and DAS28 criteria, in two other studies [22, 23]. Anderson et al. [22] analysed the RA responses to MTX vs placebo or other DMARDs alone and found disease duration to be the strongest predictor of treatment response, as RA of longer durations at treatment onset were less likely to respond to MTX. On the other hand, Hoeckstra et al. [23] found no association between disease duration and MTX response. But they did find that patients with low disease activity, according to EULAR criteria at baseline, were more likely to respond to MTX, a finding that had previously been described by others [24]. Finally, Hider et al. [25] used a large number of individual patient data from RA clinical trials, e.g. age, sex, age at diagnosis or CRP and RF levels, and found that laboratory variables collected at first consultation were poorly predictive of treatment outcome. Thus, no clear-cut relationships have been established between demographic/clinical factors and RA response to MTX. Moreover, because markers were usually evaluated after exposure to MTX, the real predictive power of these markers has not been appropriately examined.

**Pharmacogenetics**

Gene polymorphisms implicated in MTX bioavailability. Methylenetetrahydrofolate reductase (MTHFR), a folate pathway enzyme (Fig. 1), is one of the genes involved in the MTX metabolic pathway in cells that have been analysed to assess RA response to MTX. Several authors studied MTHFR polymorphisms and found that patients carrying the homozygous wild-type 677CC and/or 1298AA achieved better clinical improvement with MTX [26, 27]. However, the authors of one Japanese and two Indian studies reported contradictory findings, as they observed a MTHFR polymorphism association with efficacy [28–30]. But Kumagai et al. [28] administered a very low MTX dose (6 mg/week), which might explain the differences observed by Aggarwal et al. [29], whose patients also received folate supplementation with MTX. Thus, no clear relationship between MTHFR polymorphisms and MTX response has been established, but the diverse findings obtained probably reflect an ethnic variability and the different response criteria applied (Table 1).

Other authors studied MTX-transporter polymorphisms and RA responses to MTX, and found that patients homozygous for the reduced folate carrier (RFC) SNP 80 AA and patients with the 3435TT MDR1/ABCB1 (multi-drug resistance/ATP-binding cassette, subfamily B, member 1) genotype had better responses to MTX (Fig. 1) [31, 32]. In contrast, Takatori et al. [33] reported that 3435TT ABCB1 carriers were significantly more frequently non-responders than those with the 3435CC polymorphism. These observations suggest that genetic variations in transporters, like RFC-1 and ABCB1, may affect the RA response to MTX, even though discrepancies are probably also attributable to ethnic variability (Table 1).

A single genetic locus would probably be unable to adequately predict the response to MTX, which acts on several metabolic pathways in a polygenic disease like RA. A composite of multiple ‘risk’ loci would be more likely to identify MTX responders. Indeed, Dervieux et al. [34] reported that RA patients with higher mutation indexes in 6-aminoimidazole-4-carboxamide ribonucleotide transformylase (ATIC), thymidylate synthase-enhancer region (TSER) and RFC responded better to MTX. On the other hand, Takatori et al. [33] found no significant differences in MTX sensitivity among the patients carrying RFC1 G80A, ATIC C347G or a 6-bp deletion polymorphism of the thymidylylate synthase gene, TYSM. In addition, Wessels et al. [35] found that patients carrying the adenosine monophosphate deaminase 1 (AMPD1) 347T allele, ATIC 347CC or ITPA 94CC (inosine triphosphate pyrophosphatase) were more likely to have a good clinical response and the likelihood of a good clinical response was increased when all three favourable genotypes were present. Finally, they established a model for predicting MTX efficacy in RA patients by including AMPD1, ITPI and ITPA genotyping [36]. Genes affected by MTX and its metabolites seem to be implicated in the RA response to MTX but, to date, no consensus has been reached. A combination of multiple putative markers would probably be the most appropriate way to predict RA response to MTX, but further studies are needed to prove that hypothesis.

**Other gene polymorphisms.** Toluoso et al. [37] analysed **IL-1** gene polymorphisms to assess the influence of **IL-1β** and
IL-1 receptor antagonist (IL-1RN) genes on the RA response to MTX. They observed a higher frequency of the rare allele IL-1RN*3 in non-responders than responders. Ali et al. [38] investigated the frequency and distribution of several HLA-DR and HLA-DQ genotypes in Pakistani RA patients treated with MTX. Their results showed that only HLA-DRB1*03 was significantly more common among non-responders.

Thus, among all the studies devoted to the search for predictive biomarkers of the RA response to MTX, pharmacogenetics seems to be the only tool able to provide relevant information, even though no definitive indicators have been described to date. Future research would focus on the building of model including demographic/clinical factors and pharmacogenetics data as shown in the Wessels et al. study [36]. Indeed, they elaborated and validated a scoring system ranging from 0 to 11 with the following data: sex, RF and smoking status, the DAS and four polymorphisms in the AMPD1, ATIC, ITPA and MTHFD1 genes. Scores of ≥ 4 predict good response to MTX while scores of ≥ 6 predict no response to MTX. This study proves that such combination may lead to better-tailored initial treatment decisions in RA patients.

Markers of RA responses to biologic–MTX combinations

Clinical markers

Alessandri et al. [39] found no difference between baseline serum RF levels of responders and non-responders to infliximab–MTX but that study included very few patients. In contrast, Bobbio-Pallavicini et al. [40] examined different RF isotypes and found that high pre-treatment IgA-RF levels (130.4 vs 24.8 U/ml, P = 0.003) were associated with poor clinical responses to TNF-α inhibitors (infliximab–MTX, etanercept alone or with MTX, and adalimumab alone or with MTX). Otherwise, low baseline levels of anti-CCP antibodies seemed to be associated with better clinical responses to infliximab–MTX or infliximab–DMARD (including MTX) [40, 41]. Concerning CRP levels, Buch et al. [42] found that the majority of non-responders

FIG. 1. MTX effects on the folate pathway. After entering cells through an active transport mechanism, MTX’s main action is to inhibit the folate pathway. Once inside the cell, it is converted into its polyglutamate form (MTX-PG), which retains MTX within the cell for long periods. MTX-PG blocks dihydrofolate reductase (DHFR), which mediates the conversion of tetrahydrofolate (THF) into inosine monophosphate (IMP) and TYMS, which is required for de novo pyrimidine biosynthesis. Each enzyme directly or indirectly implicated in that synthesis or the proteins involved in MTX bioavailability are putative targets for MTX pharmacogenetics analysis. ABCB1 and ABCC1: ATP-binding cassette transporters; AICAR: adenosine monophosphate; ATIC: adenosine monophosphate deaminase; DHF: dihydrofolate; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; FPGS: folylpolyglutamyl synthase; GGH: γ-glutamyl hydrolase; methyleneTHF: 5,10-methylenetetrahydrofolate; methylTHF: 5-methyltetrahydrofolate; RFC: reduced folate carrier 1.

### Table 1. Biomarkers identified by pharmacogenetics analyses to predict RA response to MTX

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number</th>
<th>Ethnicity</th>
<th>Gene polymorphism</th>
<th>Judgement criterion</th>
<th>Polymorphism-associated response</th>
</tr>
</thead>
<tbody>
<tr>
<td>[26]</td>
<td>–</td>
<td>European</td>
<td>677CC/1298AA MTHFR</td>
<td>DAS28</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[27]</td>
<td>205</td>
<td>European</td>
<td>677CC/1298AA MTHFR</td>
<td>DAS44</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[28]</td>
<td>167</td>
<td>Asian</td>
<td>677CC/1298AA MTHFR</td>
<td>CRP level</td>
<td>Not associated</td>
</tr>
<tr>
<td>[29]</td>
<td>150</td>
<td>Indian</td>
<td>677CT MTHFR</td>
<td>DAS28</td>
<td>Not associated</td>
</tr>
<tr>
<td>[30]</td>
<td>34</td>
<td>European</td>
<td>677CT/1298AC MTHFR</td>
<td>ACR20 (6 months)</td>
<td>Not associated</td>
</tr>
<tr>
<td>[31]</td>
<td>–</td>
<td>–</td>
<td>G80AA RFC-1</td>
<td>VAS</td>
<td>Not associated</td>
</tr>
<tr>
<td>[32]</td>
<td>92</td>
<td>European</td>
<td>3435TT ABC1</td>
<td>TJC, morning stiffness</td>
<td>Not associated</td>
</tr>
<tr>
<td>[33]</td>
<td>124</td>
<td>Asian</td>
<td>3435TT ABC1</td>
<td>MTX dose maintenance</td>
<td>Not associated</td>
</tr>
<tr>
<td>[34]</td>
<td>126</td>
<td>European</td>
<td>AMPD1/ATIC/ITPA</td>
<td>DAS28</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[35]</td>
<td>126</td>
<td>European</td>
<td>AMPD1/ATIC/ITPA</td>
<td>–</td>
<td>Yes, non-responders</td>
</tr>
<tr>
<td>[36]</td>
<td>91</td>
<td>Indian</td>
<td>HLA-DRB1*03</td>
<td>–</td>
<td>Yes, non-responders</td>
</tr>
</tbody>
</table>

ABCB1: ATP-binding cassette, subfamily B, member 1; ACR: ACR criteria for improvement; AMPD1: adenosine monophosphate deaminase 1; ATIC: 6-aminomidazole-4-carboxamide ribonucleotide transformylase; IL1-RN: IL-1 receptor antagonist; ITPA: inosine triphosphate pyrophosphatase; MDR1: multi-drug resistance 1; RFC: reduced folate carrier; TJC: tender joints count; VAS: visual analogue scale.
to infliximab could be identified at treatment week 2 based on undiminished CRP concentrations, but the predictive value of CRP was not assessed, as they sought an association between changing CRP levels under infliximab. In contrast, Wolbink et al. [43] observed that patients with low, intermediate or high pre-treatment CRP levels had similar clinical responses to infliximab–MTX from 0 to 14 weeks. In addition, Morozzi et al. [44] reported that patients with serum COMP levels below the baseline decision point of 10 U/l achieved rapid and high ACR70 responses to adalimumab, and suggested that their baseline of 10-U/l threshold could be used to identify rapid ACR70 responders to that biotherpay. However, so far, no other study has confirmed that finding. Although we determined pre-infliximab–DMARD (including MTX) serum levels of numerous RA markers (IgA-, IgG- and IgM-RF isotypes, anti-CCP, six different autoantibodies, CRP, metalloproteinases-1 and -2, vitamins A and E, selenium, and cartilage and bone markers), none was able to predict response [45].

**TNF-α**

Considering the major role of TNF-α in RA pathogenesis and the benefits obtained with its inhibitors in this setting, one can hypothesize that TNF-α-mRNA quantification in peripheral blood of RA patients might provide an informative indicator of the anti-TNF response [46]. Marotte et al. [47, 48] found that circulating TNF-α bioactivity, assessed as the ability of TNF-α to stimulate synoviocyte IL-6 production, was higher in infliximab–MTX responders, thereby suggesting that high TNF-α producers were more likely to respond to anti-TNF agents. But Pachot et al. [49] showed that the TNF-α mRNA reduction, observed in the peripheral blood of RA patients after 4 h and 22 weeks of infliximab–MTX, was associated with high baseline TNF-α mRNA expression but not the therapeutic response. Hence, there is no evidence if circulating TNF-α quantification is able to predict the response to anti-TNF agents.

It is difficult to draw a conclusion concerning the abilities of clinical RA markers and TNF-α levels to predict responsiveness to biologic–MTX, in light of the conflicting results that have been published. None of the biological markers of RA clearly surpassed the others in determining the RA response at a group level, and even less at an individual level. The failure to identify any biological predictors of the RA response suggests that other as yet unknown factors probably contribute to predicting the therapeutic response.

**Pharmacogenetics**

**TNF-α polymorphisms.** Concerning the relationship between TNF-α polymorphisms and the RA therapeutic response, the G→A polymorphism at position –308 in the TNF-α promoter has certainly been the most extensively studied. Several authors found that –308 TNF-α genotyping could be a useful tool for predicting response to infliximab alone or with MTX, etanercept alone or with MTX or adalimumab–MTX [50–53] (Table 2). However, conflicting results have been reported. Some authors [54–58] found no relationship between –308 TNF-α and the responses to infliximab–MTX or etanercept alone or with MTX, including Marotte et al. [55] who studied nearly 200 RA patients. Other polymorphisms, for example, –238 or –857 in TNF-α or in the TNF receptor and biologic–MTX responses were also investigated, but no clear conclusion could be drawn [55, 58–61].

To date, no polymorphism has been unequivocally associated with a specific response to a well-defined therapeutic agent. But it has become eminently clear that TNF-α polymorphisms can influence RA responses and their analysis could be a novel approach to discovering predictive biomarkers of responses to anti-TNF-α. Among the many published results concerning TNF-α polymorphisms and treatment response, position –308 genotyping seems to be the most relevant, despite heterogeneous findings. Indeed, four studies reporting an association of the TNF-308GG genotype with better response to anti-TNF-α blocking agents were conducted with a low number of patients (less than 90) (Table 1). On the contrary, four studies involving larger sample sizes (more than 120 RA patients) did not confirm these results. These discrepancies could be explained by the size samples, the primary end point chosen and the delay of efficacy for these primary end points among studies. However, if the –308 polymorphism were to be considered a predictive marker of the RA response to anti-TNF-α [50, 53], it could not be a specific indicator of response to a given TNF-blocking agent. This distinction is critically important, because –308 TNF-α

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**Table 2. Candidate biomarkers as predictors of RA response to a biologic alone or combined with MTX**

<table>
<thead>
<tr>
<th>Analyses Reference</th>
<th>Number</th>
<th>Ethnicity</th>
<th>Treatment</th>
<th>Gene polymorphism(s)</th>
<th>Judgement criteria</th>
<th>Polymorphism-associated response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacogenetic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[50–52]</td>
<td>59/22/63</td>
<td>European</td>
<td>INF–MTX</td>
<td>–308GG TNF-α</td>
<td>DA528 W22, 8 or 24</td>
<td>Yes, responders</td>
</tr>
<tr>
<td></td>
<td>78/198/20</td>
<td>European</td>
<td>INF–MTX</td>
<td>–308GG TNF-α</td>
<td>HAQ, VAS, CRP W12 or ACR20–70</td>
<td>Not associated</td>
</tr>
<tr>
<td>[52]</td>
<td>13</td>
<td>European</td>
<td>ETA–MTX</td>
<td>–308GG TNF-α</td>
<td>DA528 W24</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[53, 57, 58]</td>
<td>86/123/457</td>
<td>European</td>
<td>ETA–MTX</td>
<td>–308GG TNF-α</td>
<td>ACR20–70, DA528 W6/W12/M12</td>
<td>Not associated</td>
</tr>
<tr>
<td>[55]</td>
<td>198</td>
<td>European</td>
<td>INF</td>
<td>–238–308 TNF-α</td>
<td>ACR20–70, DA528 W30</td>
<td>Not associated</td>
</tr>
<tr>
<td>[58]</td>
<td>70</td>
<td>South American</td>
<td>ETA–MTX</td>
<td>–857CT TNF-α</td>
<td>ACR50, R20, W12</td>
<td>Not associated</td>
</tr>
<tr>
<td>[59, 57, 58]</td>
<td>457</td>
<td>North American</td>
<td>ETA</td>
<td>TNF-α R1/R1</td>
<td>ACR50, M12</td>
<td>Not associated</td>
</tr>
<tr>
<td>[60]</td>
<td>80</td>
<td>European</td>
<td>ADA–MTX</td>
<td>+4845 IL1-β</td>
<td>SJC</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[60]</td>
<td>126</td>
<td>European</td>
<td>INF–MTX</td>
<td>IL1RN*2</td>
<td>ACR20, DA528 W12</td>
<td>Not associated</td>
</tr>
<tr>
<td>[60]</td>
<td>123</td>
<td>European</td>
<td>ETA–MTX</td>
<td>SE</td>
<td>ACR20, DA528 W12</td>
<td>Not associated</td>
</tr>
<tr>
<td>[61]</td>
<td>457</td>
<td>North American</td>
<td>ETA–MTX</td>
<td>Two SE copies</td>
<td>ACR20–70 M12</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[62]</td>
<td>182</td>
<td>European</td>
<td>ADA–MTX</td>
<td>Number of SE copies</td>
<td>ACR50</td>
<td>Not associated</td>
</tr>
<tr>
<td>[63]</td>
<td>292</td>
<td>European</td>
<td>INF/ETA–MTX</td>
<td>15S/V/F FC/rIIA</td>
<td>ACR50–70, EUPLAR</td>
<td>Not associated</td>
</tr>
<tr>
<td>[64]</td>
<td>30</td>
<td>North American</td>
<td>INF/ETA/ADA</td>
<td>FC/α/β/α</td>
<td>TJC, SJC, ESR, CRP</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>Transcriptomic</td>
<td>[65]</td>
<td>European</td>
<td>INF–MTX</td>
<td>41 transcripts</td>
<td>DA528 W12</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[66]</td>
<td>33</td>
<td>European</td>
<td>INF–MTX</td>
<td>41 transcripts</td>
<td>DA528 W12</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[67]</td>
<td>32</td>
<td>European</td>
<td>ADA–MTX</td>
<td>51 transcripts</td>
<td>DA528 W12</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[70]</td>
<td>19</td>
<td>European</td>
<td>ETA–MTX</td>
<td>42 transcripts (Day 3)</td>
<td>DA528X-ray</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[71]</td>
<td>10</td>
<td>European</td>
<td>INF–MTX</td>
<td>279 transcripts</td>
<td>DA528 W12</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>[72]</td>
<td>18</td>
<td>European</td>
<td>INF–MTX</td>
<td>189 transcripts</td>
<td>DA528</td>
<td>Yes, responders</td>
</tr>
<tr>
<td>Proteomic</td>
<td>[73]</td>
<td>European</td>
<td>INF–MTX</td>
<td>Four and two proteins</td>
<td>ACR20, ACR70</td>
<td>Yes, responders</td>
</tr>
</tbody>
</table>

ACR: ACR criteria for improvement; ADA: adalimumab; ESR: ESR/1st hour; ETA: etanercept; INF: infliximab; M: month; SJC: swollen joints count; TJC: tender joints count; VAS: visual analogue scale; W: week.
genotyping would not be useful to discriminate among patients, since some respond better to one anti-TNF agent than another [8].

**Other cytokine polymorphisms.** Comparing responders to non-responders, Camp et al. [62] found a highly significant association between carriage of the rarer allele at +4845 IL-1α and response to anakinra and a weaker association for +3954 IL-1β. Tolusso et al. [37] reported that patients with the IL-1RN*2 allele responded better to infliximab. Further studies are needed to confirm these observations and determine whether or not a real association exists with treatment response. Multiple cytokine polymorphisms have also been studied. Padyukov et al. [57] observed that a given association of allelic forms influencing TNF (−308 TTN1/TNF1) and IL-10 (−1087G/G) production was associated with good responses to etanercept alone or with another treatment including MTX, according to ACR20 and DAS28 criteria, whereas another combination of allelic forms influencing TGF-β1 (the rare C allele in codon 25) and IL-1Ra (A2 allele) production was associated with poorer responses.

**MHC gene polymorphisms.** Notably, the TNF-α locus is in close proximity to the HLA-B and HLA-DR genes on chromosome 6, and can occasionally interfere with the latter. HLA-DRB1 alleles contain a similar sequence at the amino acid position 70–74 in the third hypervariable region of the DR molecule (called the ‘shared epitope’ or SE), which is strongly associated with RA and could be implicated in its response to treatment [63]. Several authors investigated potential relationships between the HLA-DRB1 locus and/or SE alleles in terms of therapeutic response and found no influence of SE frequency on the response to infliximab–MTX, etanercept alone or with another treatment including MTX, adalimumab alone or adalimumab–DMARD (including MTX) [54, 55, 57, 59]. In contrast, Criswell et al. [58] found that the presence of two SE copies was significantly associated with the response to etanercept.

Although an HLA-DRB role in RA is well known, a possible association between it and treatment response has not yet been definitively demonstrated.

**Fc-receptor polymorphisms.** Other than their cytokine-neutralizing properties, anti-TNF biologics may exert their actions via their IgG1 Fc fragment and bind to cellular Fcγ receptors (FcγR). Studies on FcγR polymorphism could provide predictive markers of interest. Based on a homogeneous population of 282 RA patients, Kastbom et al. [64] concluded that the FcγRIIA-158VF polymorphism was an unlikely candidate to influence the responses to infliximab–MTX and etanercept–DMARD (including MTX). In addition, Miceli-Richard et al. [65] noted that FcγRIIA/IIIB were not associated with RA responses to adalimumab alone or with MTX. Unlike those findings, Tutucu et al. [66] found that RA patients with the FcγRIIA-158FF genotype responded better to one of the three anti-TNFs than those carrying at least one V allele. But the latter results were not completely convincing because of the very small number of patients with arthritis, and not exclusively RA, enrolled and they represented four ethnic groups (white, Hispanic, African American and Asian) with dissimilar genetic backgrounds.

Therefore, all currently available pharmacogenetics data clearly show that genetic factors could influence therapeutic responses (Table 2). However, at present, no unanimous results or consensus would allow a clear-cut conclusion to be drawn about genotype–treatment response relationship(s). And, it is even more difficult to establish a clear relationship because treatment responses were not uniformly evaluated in all the studies, which makes their comparison difficult (Table 2). In addition, the ethnic differences among patients investigated would probably have a supplementary impact on the influence on treatment response. The discrepancies among results could also be imputable due to the difference in biological targets (MTX, infliximab, etanercept, etc.), too small sample sizes and error due to multiple comparisons. Concerning underpowered studies, poorly reproducible results remain unavoidable. In the future, the number of patients included in pharmacogenetics investigations must be adequate to avoid false positive results. Moreover, major efforts concerning the standardization of selected patients should be done to find widespread application in clinical practice. Finally, genome-wide studies could be more powerful than restricted polymorphisms studies to improve the identification of putative biomarkers able to predict response to biologic agents. Recently, some authors carried out a genome-wide association study and provided a list of new candidate SNPs (MAFB, INFK, PON1) which need to be validated by further pharmacogenetic investigations [67]. In addition, other mechanisms may be involved, as genetic polymorphisms do not explain why some patients respond to one TNF-α antagonist but not to the others [8].

**Pharmacogenomics**

**Transcriptomic analyses.** Our group used microarrays to analyse peripheral blood mononuclear cell (PBMC) transcriptomes of RA patients before treatment with either infliximab–MTX or anakinra–MTX [68, 69], and identified and validated two combinations of 41 and 51 transcripts, respectively, expressed as a function of the therapeutic responses of the patient sets. Although those observations must be confirmed with larger patient cohorts, our study model enabled the identification of combinations of transcripts able to predict responses to these biotherapies. This procedure is a non-invasive and its biological material (PBMCs) is easily obtained for the screening of markers predictive of an individual patient’s therapeutic response. Toonen et al. [70] independently assessed the power estimation of our expression profiles and found that, based on the number of samples analysed, the standard deviation was sufficiently powered.

Koczan et al. [71] identified 42 genes in PBMCs with comparable baseline expression levels that were differentially regulated in responders and non-responders after only 3 days of treatment. Expression profile changes of seven pairs and 10 triplets within those 42 genes on Day 3 were found to have >89% prediction accuracy. Even though the sets of genes (pairs or triplets) were unable to predict response before etanercept–DMARD (including MTX) administration, they clearly demonstrated that it was possible to predict response very early during treatment. That study was, unfortunately, limited by the use of the same patient set to validate their gene sets.

Using gene expression profiles of synovial biopsies, Lindberg et al. [72] identified 279 and 382 differentially expressed genes when non-responders to infliximab–MTX were compared with, respectively, good responders and moderate/good responders. However, that investigation recruited only 10 patients, probably because synovial tissue is often difficult to obtain, which is unusual for microarrays studies. Indeed, these analyses require high numbers of patients to discern relevant genes and generate significant data. However, it was a pilot study trying to demonstrate the feasibility of using synovial tissue gene-expression profiling to predict therapeutic responses. Similarly, van der Pouw Kraan et al. [73] applied an arbitrary threshold of 1.4-fold expression difference between infliximab–MTX responders and non-responders and identified 189 synovial genes which indicated that responders had higher levels of cellular and inflammatory activities. No validation set of patients was used in that study, once again probably owing to the difficulty of obtaining synovial tissue.

The rare overlap of genes identified in transcriptomic studies aiming to predict therapeutic responses does not question the ability of identified combinations to predict responses, as the goal was not to determine a pathophysiological process (Table 2). However, more studies with many more patient samples
are needed to confirm the validity of a predictive combination. Moreover, future research focusing on the identification of predictive markers will be performed with pan-genomic platforms in order to improve the combination to be powerful and increase the effectiveness of prediction test.

Proteomic analyses. As for transcriptomic studies, recent advances in proteomics technology might be extended to include the identification of markers able to predict therapeutic responses [74]. But at the present time, very few studies have been published (Table 1). Trocmé et al. [75] identified two distinct protein profiles from sera (with two distinct arrays) that accurately reflected responses to infliximab–MTX before starting treatment: a small 3.9-kDa protein overexpressed in non-responders and a combination of four proteins, three, at 7.8, 8 and 74 kDa, which were significantly more abundant in non-responders, and the fourth, at 28 kDa, which was overexpressed in responders. Drynda et al. [76] examined SF and plasma samples. They found that, after 3 months on etanercept, the plasma concentration of the S100A8–A9 heterocomplex of the small calcium-binding proteins was significantly lower in responders and, thus, considered it a discriminatory marker of RA SF that could also be a useful marker for monitoring anti-TNF-α efficacy. Although that study had not been designed to find predictive factors, in light of the few published results obtained with this experimental approach, these findings enhance its promise and open the way for the use of proteomics to identify predictive biomarkers of therapeutic responses. Huerer et al. [77] conducted proteomic analyses of serum proteins and identified a panel of secreted molecules, including fibrinogen, calpastatin, HSP, and proteoglycans, which correctly predicted that 73% of the patients would subsequently respond to etanercept.

Even though few studies concerning biomarkers predictive of the RA response to biologic–MTX combinations have been published, their results support proteomics as a promising tool to look for such indicators.

Conclusion

The clinical efficacy of anti-TNF and anti-IL-1 agents is well-established, but some patients do not respond to them. After >10 years, anti-TNF-α and anakinra are now routinely prescribed to certain patients but clinicians still lack markers for daily use to predict their responses to these immunotherapies. Rheumatologists require well-founded guidelines to orient their choices of immunotherapy for individual patients. Herein, we reviewed putative candidates able to predict therapeutic responses. Most studies concerned anti-TNF-α and a few tested anakinra but, to date, neither rituximab nor abatacept has been investigated with this objective. Published results of pharmacogenetics and pharmacogenomics analyses of MTX alone or combined with a biologic have supported their promising abilities to identify biomarkers predictive of therapeutic responses at the individual level. However, data from these studies must be confirmed before clear-cut conclusions can be drawn. So, at present, no robust biomarker exits for rheumatologists to use in routine clinical practice to predict responsiveness to MTX and/or biologics.

The most relevant biomarker predictive of therapeutic response will probably not be a single factor but rather a combination of several markers identified by large-scale gene and protein prospecting with both technologies. A prediction model of response to treatment could then be established with the resulting data processed with algorithms and decisional trees. Soon, other new molecules will become available to treat RA patients. Also, more the new agents are available, the more effective RA treatment will be, and also the more clinicians will require precise markers predictive of responses to avoid unnecessary risks and expenditures in case of failure. Moreover, a new category of indicators would rapidly become essential as the new goal of therapeutic recommendations would not be merely a good response to the biologic but RA remission. The importance of identifying such biomarkers predictive of therapeutic responsiveness is further underlined by the fact that the public health agencies are trying to establish guidelines for their validation.

Once validated, proteomic and transcriptomic analyses can become part of routine laboratory screening with major advantages: rapid prediction of therapeutic efficacy protects the patient against unnecessary drug exposure, unproductive loss of time and potential side effects, while sparing health care budgets from expenditures for iatrogenic illnesses and ineffective costly treatments. So, fast tracking of such a research is a high priority for future efforts.

Rheumatology key messages

- Large-scale gene and protein analysis approaches can identify new putative markers predictive of therapeutic responses.
- Relevant biomarkers predictive of therapeutic responses will probably be a combination of several markers.

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