Review paper

Reumatologia 2016; 54, 4: 177–186
DOI: 10.5114/reum.2016.62472

Personalized medicine in rheumatology

Anna Kłak[1], Agnieszka Paradowska-Gorycka[2], Brygida Kwiatkowska[3], Filip Raciborski[1]

[1]Department of Gerontology and Public Health, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland
[2]Department of Biochemistry and Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland
[3]Clinic of Early Arthritis, National Institute of Geriatrics, Rheumatology and Rehabilitation, Warsaw, Poland

Abstract

In the era of the 21st century, rheumatoid arthritis (RA) is still poorly characterized. Rheumatoid arthritis is a common but heterogeneous disease, not only in the course and clinical symptoms, but also in the clinical response to treatment. Now it is known that early, correct diagnosis and starting treatment with disease-modifying drugs (DMARDs), of which methotrexate (MTX) remains the gold standard in the treatment of RA, is crucial in order to prevent joint destruction, functional disability and an unfavourable disease outcome. Early diagnosis of rheumatoid arthritis is significant in so much as the primary treatment can be started better. Pharmacogenetic and pharmacogenomic studies, which help determine the genetic profile of individual patients, may bring us closer to personalized medicine. Further studies on RA should allow for the identification of disease-specific genes at the stage when their tolerance by the organism is still preserved (before auto-aggression develops).

Key words: personalized medicine, rheumatoid arthritis, biomarkers, genetic test.

Introduction

The basis of future medicine is a heterogeneous disease pattern which includes its causes, dynamics, course as well as response to treatment. The main rule of personalized medicine is the conviction that the same disease can have a different cause, course or therapeutic efficiency, depending on the patient. Therefore an individual approach to each patient is crucial [1].

In times of unprecedented scientific and technological breakthroughs, personalized medicine that focuses on molecular diagnostics as well as on determining the risk of morbidity makes it possible to tailor the treatment to the patient's individual needs and, therefore, to improve safety, effectiveness and the costs of the treatment. Personalized medicine, based on clinical, genetic, genomic and environmental data, unique for each patient, is the opposite of the traditional therapeutic process that is based on adjusting the treatment to visible symptoms of the disease. Personalized medicine is based on tailoring the medicine in an appropriate dose to an individual patient at an appropriate time. This approach is possible thanks to the molecular analysis, not only of particular diseases, but also individual patients. Moreover, the therapy preceded by pharmacogenetic tests is more effective as it allows for the selection of medicine based on a specific target. Therefore the response of the patient's organism to the implemented treatment is foreseeable. Increased effectiveness constitutes one advantage as well as reduced risk of side-effects [2]. Undoubtedly, saved time and reduced cost of treatment constitute additional benefits [3].

The concept of personalized medicine assumes that the identification of the disease at the molecular level makes it possible to introduce the treatment in patients who are still in good health. Due to the results of genetic tests, an individual predisposition to develop a given disease can be predicted. The management of the disease depends on the correlation of two factors: genetic determination and the increased or reduced risk of disease. Preventive therapies should be therefore introduced in the group of patients with high risk of disease. Preventive care should consist of change in the patient's lifestyle (in order to eliminate bad habits) as well as period-

Address for correspondence:
Agnieszka Paradowska-Gorycka, Department of Biochemistry and Molecular Biology, National Institute of Geriatrics, Rheumatology and Rehabilitation, Spartanska 1, 02-637 Warsaw, Poland, e-mail: paradowska_aga@interia.pl
Submitted: 22.07.2016; Accepted: 12.08.2016

ic tests (in order to detect the disease at the preclinical stage) [4, 5].

Traditional treatment in rheumatoid arthritis

The traditional model of treatment of RA is based on pharmacological treatment, rehabilitation, education and psychotherapy. The objective of the treatment is to eliminate the pain, limit or stop the inflammation, maintain proper functioning of the locomotor system, including slowing down or stopping structural changes in joints, as well as to prevent organ alterations.

Pharmacological treatment of RA should start as soon as possible, preferably within 6–12 weeks after manifestation of the first symptoms, and should be effective, i.e. lead to remission of the disease. The time factor, referred to as the therapeutic window (a maximum of 12 weeks after manifestation of the first symptoms) is the strongest predictor of remission [6]. According to the recommendations of the European League Against Rheumatism (EULAR) of 2013, patients with active RA should be monitored every 3 months, and the change in treatment in the case of ineffectiveness should occur no longer than after 6 months of therapy.

In the treatment of RA synthetic disease-modifying antirheumatic drugs (sDMARDs) and biological disease-modifying antirheumatic drugs (bDMARDs) are the most important. Their task is to stop further development of the disease. Drugs which affect the symptoms of the disease but do not inhibit the progress of the disease include nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids (GCs) and analgesics. The regime of pharmaceutical treatment of rheumatoid arthritis is presented in Figure 1 [7].

A drug of the next generation is tofacitinib. It is used in the treatment of active rheumatoid arthritis in symptom severity from moderate to severe. Although this drug is available in 20 countries, including Canada, Argentina, Japan, Switzerland, Russia and Turkey, it is still waiting for approval from the European Medicines Agency (EMEA). Acceptance is required before tofacitinib can be used in EU countries including Poland.

Nonsteroidal anti-inflammatory drugs

Drugs in this group quickly reduce the symptoms of the disease, i.e. the pain and the duration of morning stiffness. The medicine should be tailored to each patient, depending on the risk of cardiovascular or gastrointestinal complications, according to European Recommendations of 2011 [8].

Glucocorticoids

According to EULAR recommendations of 2010, glucocorticoids (GCs) are recommended as a first-line therapeutic strategy, together with DMARDs. They are administered at low doses (< 10 mg/day) and for a short

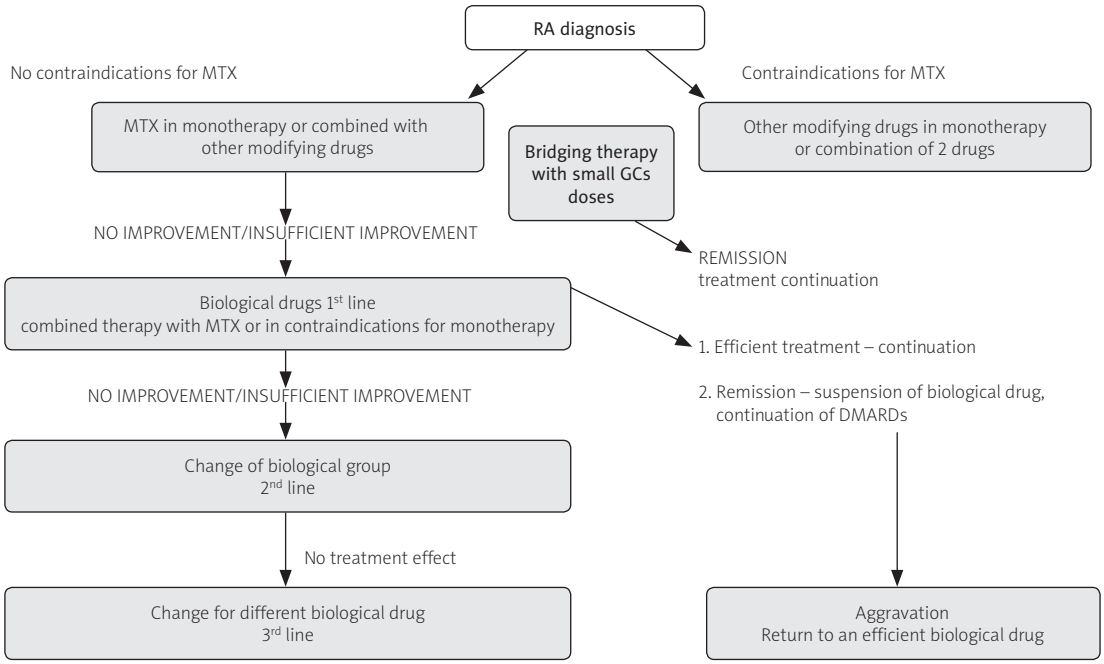

Figure 1. The regime of pharmaceutical treatment of RA.
Source: Own work based on EULAR recommendations [7].

period of time at higher doses in the case of disease aggravation [9]. It should be however noted that according to recent findings chronic use of glucocorticoids in RA increases the risk of heart attack by 68%; therefore the necessity to administer them should be assessed individually for each patient. The risk of heart attack after GCs therapy depends on the dose – it increases by 13% with the increase of dose by 5 mg as well as on the duration of use – it increases by 10% each year [10]. According to EULAR recommendations of 2013, for the management of rheumatoid arthritis it is recommended to take glucocorticoids in small doses at the onset of the disease, together with DMARDs for no longer than 6 months, if possible [10].

Pain treatment and analgesics

According to the recommendations of international specialists in the area of rheumatology from the 3E Initiative (Evidence, Expertise, Exchange) in patients suffering from arthritis, the pain should be routinely measured using validated scales, such as the Visual Analogue Scale (VAS), the Numerical Rating Scale (NRS), and the Verbal Rating Scale (VRS). For patients with arthritis, paracetamol is recommended in the case of chronic pain or paracetamol combined with nonsteroidal anti-inflammatory drugs. Patients with inflammatory rheumatic diseases can also be treated with tricyclic antidepressants and neuromodulators that influence the reception of pain stimuli. Drugs that decrease the muscle tone, called muscle relaxants, as well as benzodiazepines are not recommended. Weak opioids can be used for a short period of time when current therapy is not effective. Long-term use of weak opioids can be considered, but then the therapy requires regular supervision. Strong opioids (morphine and its derivatives) should be used only in exceptional cases [11].

Disease-modifying antirheumatic drugs

Synthetic disease-modifying antirheumatic drugs

Synthetic disease-modifying antirheumatic drugs include methotrexate (MTX), sulfasalazine (SSZ), leflunomide and, in exceptional cases, azathioprine, cyclosporin A and cyclophosphamide.

Methotrexate is a drug used in the first-line therapeutic strategy for RA and should be recommended immediately after diagnosis. The effective dose of MTX is 20–30 mg once a week. The starting dose is 10–15 mg and should be increased by 5 mg every 2–4 weeks, until it reaches 20–30 mg. Methotrexate acts by inhibiting dihydrofolate reductase and thus reduces the amount of tetrahydrofolate. It inhibits the synthesis of nitrogenous bases such as thymidine as well as purines and pyrimidine metabolism. Methotrexate also inhibits cell proliferation, increases T cell apoptosis and the level of endogenous adenosine, and alters expression of intercellular adhesion molecules, which impacts the inhibition of pro-inflammatory cytokine production and cellular response. Methotrexate inhibits inflammatory function of neutrophils, macrophages, monocytes and dendritic cells.

The most common side effects of MTX include the increase in aminotransferases activity (10–43%), gastrointestinal symptoms (20–65%), stomatitis (10–15%), anaemia (10–15%), leukopenia (12%) and thrombocytopenia (12%). Dysfunction of the central nervous system (8– 10%), hair loss (8%), pneumonia (2.1–8%), infections (5%) and subcutaneous nodules (2–6%) are observed less often [12].

Methotrexate hepatotoxicity increases in elderly patients with RA and depends on therapy duration. Benign hepatic fibrosis occurs in ca. 7% of patients, whereas cirrhosis occurs only in 0.1% of patients. The following tests should be performed before MTX therapy: liver transaminases (AST, ALT), creatinine, albumin, blood count and blood smear. Hepatitis B and hepatitis C infection should be excluded; therefore HBsAg and HCV antibody tests should also be carried out as well as chest X-ray. HIV testing, glucose concentration, lipid profile and pregnancy test could also be considered. Control tests should also be carried out during MTX therapy (AST, ALT, blood count and blood smear, creatinine concentration), initially every 4–6 weeks and after achieving the target dose every 1–3 months. Women in their reproductive years must use an effective contraceptive method because MTX is teratogenic. The therapy must be discontinued 3 months prior to the planned pregnancy – both in women and men. Methotrexate cannot be used by pregnant or breast-feeding women [13].

Sulfasalazine is recommended in rheumatoid arthritis therapy when MTX cannot be used or in combination therapy. Sulfasalazine is split in the colon by bacteria into two main metabolites: sulfapyridine and mesalazine (5-aminosalicylic acid). The mechanism of SSZ action in the treatment of RA is still not well known. Current knowledge suggests that it inhibits the production of antibodies in response to stimulation with antigens and reduces the expression of cell adhesion molecules in leukocytes and epithelial cells. The therapeutic dose in RA is 2–4 g/day.

Leflunomide is a prodrug the activity of which depends on an active metabolite (A771276) created in the intestinal walls and liver as a result of metabolism. The half-life of A771276 is around two weeks. Leflunomide works by inhibiting the production of interleukin 2 (IL-2), the activity of tumour necrosis factor α (TNF-α), the production of antibodies in B lymphocytes as well as the

proliferation of T lymphocytes and by reducing the migration of inflammation cells to the synovial membrane. The loading dose of leflunomide is one pill of 100 mg per day during the first three days of treatment. Then the maintenance dose of 10 to 20 mg/day is used (depending on the severity of symptoms).

Biological disease-modifying antirheumatic drugs

Biological drugs are recommended when synthetic DMARDs (MTX in particular) have proved to be ineffective and the disease remains active. Contraindications for application or side effects of DMARDs are another reason to use biological drugs.

Tumour necrosis factor α inhibitors were the first biological drugs used in treatment of rheumatoid arthritis. At present in Poland the following drugs are registered and qualified for therapeutic programmes of rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), ankylosing spondylitis (AS) and psoriatic arthritis (PsA): infliximab, etanercept, adalimumab, certolizumab pegol, golimumab.

Biological drugs with a different mechanism of action

At present biological drugs with a different mechanism of action, such as rituximab, are also available. The drug binds specifically to the transmembrane antigen CD20, which is a glycosylated phosphoprotein expressed on the surface of pre-B cells and mature B cells. The Fab fragment of a rituximab particle binds to the CD20 antigen on B cells and through the Fc region triggers the mechanisms of the immune system that lead to the lysis of B cells. Rituximab is administered intravenously in the dose of 1000 mg in two infusions every 2 weeks, and it is used in combination therapy with MTX. Its final mean half-life is 20.8 days (range 8.58 to 35.9 days).

Tocilizumab is a humanized monoclonal IgG1 antibody produced by genetic engineering that binds specifically with the interleukin-6 receptor (IL-6R), both soluble and membrane bound, which inhibits the signal transduction mediated by both mIL-6R and sIL-6R. It is administered intravenously in the dose of 8 mg/kg every 4 weeks. In patients with body weight > 100 kg a dose higher than 800 mg/infusion is not recommended. Tocilizumab can be used in monotherapy as well as in combination therapy with MTX. The half-life of each dose of 8 mg/kg every 4 weeks is 8 to 14 days.

Abatacept is a recombinant soluble fusion protein that consists of an extracellular fragment of antigen 4 bound to a human T cell (CTLA-4), fused to the modified Fc region of the immunoglobulin IgG1. Abatacept inhibits CD80 and CD86 molecules by binding to CD80/86 receptors on the surface of antigen cells, which causes modelling of the stimulating impact of CD28 protein on T cells. Abatacept is produced by recombinant DNA technology in Chinese hamster ovary cells. It is administered intravenously in the dose of 10 mg/kg a month in weeks 0 and 2 and then every 4 weeks. Abatacept is used in combination therapy with MTX or other DMARDs, and its final mean half-life is about 13 days.

A new approach for the treatment of rheumatoid arthritis – the need for a new biomarkers

In the era of the 21st century, rheumatoid arthritis is still poorly characterized. RA is a common but heterogeneous disease, not only in the course and clinical symptoms, but also in the clinical response to treatment. Now it is known that early, correct diagnosis and starting treatment with DMARDs, of which MTX remains the gold standard in the treatment of RA, is crucial in order to prevent joint destruction, functional disability and an unfavourable disease outcome [14].

RA patients who fail treatment with MTX due to toxicity or lack of efficacy are switched to other therapeutic options in order to select the most advantageous. The costs and potential adverse effects associated with multiple ineffective therapies are high, and it is not always possible to achieve a satisfactory treatment efficacy. Proper selection of suitable and safe therapy may be an effective tool not only to ameliorate symptoms of the disease (pain and swelling of the joints, fatigue), but also to prevent damage to the joints, to improve the length and quality of life, and for remission of the disease. Unfortunately, although MTX and biologic agents generally improve outcomes for RA patients, up to 40–60% of RA patients fail to achieve a satisfactory response, and about 15–30% of the patients develop adverse drug events [15, 16].

The reason for this variability between individuals is unclear, but it leads to studies identifying biomarkers predictive of the treatment response. The use of traditional markers that have been clinically useful such as autoantibodies, acute phase reactants, bone and cartilage markers, and various cytokines [17] in the context of personalized medicine is insufficient. Therefore, biologists and rheumatologists believe that the identification of novel, better biomarkers would allow understanding of the molecular pathways involved in the pathophysiology of the disease and more appropriate selection of the optimal treatments for first-line care. It is probable that serum biomarkers such as protein or microRNA, and examination of gene signatures and gene expression profiles, may be more helpful than static gene arrays. We believe that the achievement of satisfactory treatment

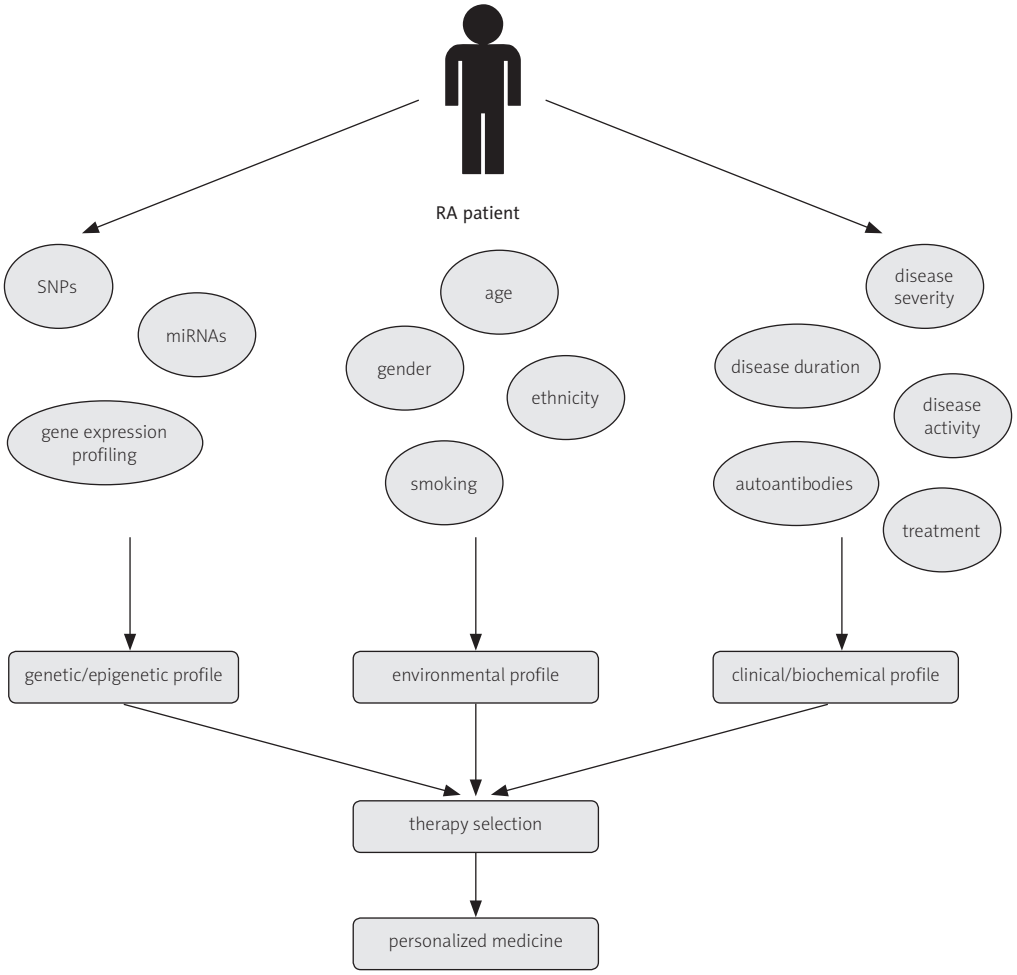

Figure 2. Procedure of therapy optimization in RA patients.

is possible only through comprehensive action to identify biochemical, clinical and genetic/epigenetic biomarkers that help us understand the variable reasons for targeted therapy (Fig. 2). Genetic/epigenetic predisposition is probably one of the most important factors that play a role in response to the efficacy and toxicity of the drug. These biomarkers may be useful in daily practice because they do not vary with time and analysis can be carried out using samples derived from patients' blood [18]. In addition, the knowledge about the molecular basis of the disease affects the clinical strategies, which are no longer a linear process, and the integrated system combining molecular and pharmacogenetic data as well as clinical information from one patient in the system, called the "knowledge management system".

Role of genetics studies – pharmacogenetics

For over 40 years it has been established that genetic variants can influence disease susceptibility, disease progression, or the individual's response to therapy [19]. Moreover, they can be very attractive biomarkers due to standardized assays and associations used to identify and validate tests, and they have a number of benefits over other markers [20]. In the last decade, many studies have demonstrated that both the toxicity and the efficacy of drugs can be modified by the presence of single nucleotide polymorphisms (SNPs) in genes involved in the metabolism, transport, and function of medications – pharmacogenetics [21–24].

The candidate gene for genetic variation analysis were selected from the genes involved in the pathogenesis of rheumatoid arthritis and/or the cytokine signalling pathway [24]. Although several SNPs have been detected to be associated with drug response in patients with RA, the majority of these findings are still inconclusive and inconsistent as well as limited to known genes involved in the DMARDs' and biologic agents' cellular pathways [21, 23]. Several factors may contribute to the inconsistency: size of samples (usually too small, $n < 1000$), number of SNPs in drug target genes, differences in ethnic popula-

tions, population stratification, genetic background for tested populations, differences in clinical characteristics of patients, differences in disease stages, previous drug history, and finally differences in study design [18, 23].

Because not a single gene but multiple genes are involved in the pathogenesis of RA as well as drug responses, genome-wide association studies (GWAS) could be a more potent approach for identifying candidate genes to include in these pharmacogenomic models [15, 22]. GWAS scan hundreds of thousands or even millions of polymorphisms across the whole genome per individual; they have proven to be a powerful hypothesis-free method to identify common disease-associated polymorphisms that are present in the general population [18, 25]. The success of GWAS has opened a wide new perspective for exploration and highlighted the complicated genomic architecture susceptibility [19]. As the GWAS do not identify the association between a gene and phenotype of the disease, findings from GWAS as well as the biological and clinical interactions between the specific loci and diseases should be further explored by traditional candidate gene studies such as allelic discrimination by TaqMan real-time PCR [18, 25]. In addition to GWAS, new techniques such as next-generation sequencing (NGS) are not limited to gene chips and thereby enable identification of common and rare variants determining the response to drugs or adverse drugs reactions [18]. NGS technology, with high sensitivity and high-bandwidth properties, results in the provision of more convincing data, which cannot be obtained using GWAS. Moreover, the analysis using NGS contributed significantly to both basic and clinical studies. Also, the discovery of causative gene loci gives us the opportunity to identify factors involved not only in the pathogenesis of RA but also correlated with drug responses. Additionally, gene identification is one of the key steps in the discovery of the pathogenesis of polygenic diseases. However, the above studies suggested that genetic polymorphisms (located in promoter, regulatory or coding sequences of the respective gene) make a substantial but incomplete contribution to the risk of RA developing. It is widely accepted that gene-expression profile and epigenetic changes provide an additional window for understanding the possible mechanisms involved in the pathogenesis of RA, and they will enable the development of new intervention strategies.

Gene expression profiling – pharmacogenomics

The idea of "personalized or precision" medicine in rheumatology, which allows for potential use of genetic information for a rational choice of therapeutic intervention, to optimize patient outcomes while minimizing side effects, is a natural evolution of developing the knowledge which we gained over the past few decades. Gene-expression profiling as an analysis of the expression or activity of genes represents molecular fingerprints that offers great potential for understanding RA aetiopathogenesis as well as for patient management and personalization of treatment decisions [16, 26].

Gene expression is typically measured in different tissues or conditions, and many human transcripts have limited expression during a particular stage of disease [18]. The abundance of a gene transcript can be directly modified by genetic variants in regulatory elements which may modulate gene expression of local genes (cis-expression quantitative trait loci (eQTL), likely acting on the same chromosome) or genes at a distance on non-contiguous chromosomes (trans-eQTL) [18, 27]. eQTLs are a powerful tool to connect polymorphisms of unknown function whose expression levels are associated with a complex trait because of pleiotropy [28, 29].

This method allows the identification of relationships between genes and regions associated with risk of RA in order to better understand the biology of RA disease, and potential paths for drugs. Considering that genes mediate their biological roles in groups rather than in isolation, genome-wide gene expression analysis with cDNA microarrays has become a powerful tool that can be used to identify genes that may be biomarkers for the diagnosis and monitoring of disease activity as well as for the prediction of clinical responses to certain anti-rheumatic treatments in RA patients [30, 31].

cDNA microarrays enabled a more detailed analysis of drug responses because thousands of genes were screened and expression levels were correlated with drug responses. Although microarray analysis has led to detection of a gene signature that differentiated the stage of RA and the response to treatment, the small size of cohorts and dynamic phenotype of RA are key obstacles to the identification of reliable biomarkers and caused that diagnostic and prognostic microarrays have not been developed and clinically applied. NGS, which has several advantages over microarrays including massive parallel sequencing of RNA and detection of non-coding transcripts and alternative splicing events, is now challenging microarrays as the tool of choice for genome analysis [16].

After all, microarrays are established tools that the research community is familiar with, plus the bioinformatics pipelines for array data analysis are mature. Microarrays may be useful as a screening tool when the DNA or RNA of large numbers of samples, such as clinical isolates, need to be probed or when either a low-cost 'quick look' is warranted. However, when samples of interest are defined, NGS could be used to provide comprehen-

sive deep-sequence analysis of genomic DNA to identify mutations [32]. Genome-wide approaches of microarray analysis and NGS may improve diagnostic accuracy, prediction of therapeutic responses and overall survival of patients with RA. Moreover, the successful translation of gene-expression profiling data into clinical use in cancer ensures strong reasons for a similar approach to improve the care of patients with rheumatic diseases.

MicroRNA profiling – pharmacogenomics

Not only genetic polymorphisms, located in the promoter, regulatory or coding sequence of the respective gene, leading to changes in expression of proteins, can affect its level and/or function. It is widely accepted that epigenetic mechanisms such as DNA methylation or microRNA (miRNA, miR) are needed to implement key functions in gene expression regulation. Moreover, recent studies have demonstrated that epigenetic anomalies are emerging as major pathogenic features of rheumatoid arthritis, and miRNAs are new pharmacogenomics biomarkers for anti-rheumatic drugs [33]. miRNAs are small, noncoding RNA molecules constituting about 1–2% of the whole genome and tightly regulated biological processes through modulation of protein expression at the posttranscriptional level [34–36].

They function as crucial regulators of immune response in both physiological and pathological conditions. Previously miRNAs were considered to act as intracellular modulators of gene expression. However, accumulating evidence has demonstrated that as miRNAs are stably present in cell-free form in body fluids such as plasma or serum and can be easily measured in tissues as well as body fluids by polymerase chain reaction (PCR) or array technology, they are becoming new candidate biomarkers for diagnosis and prognosis in various diseases including RA. In the past 2 years, miRNAs were suggested to affect the immune cell niche, and to act as modulators of cellular metabolism [35]. Abnormal miRNA expression in patients with rheumatic diseases was first reported less than a decade ago; several miRNAs were up-regulated in both plasma/serum fluids and inflamed joints [34, 35].

Moreover, they may be helpful in monitoring RA severity and understanding its pathogenesis, as the miRNAs can be apparently expressed even at different stages of disease progression [33]. Specifically, several miRNAs have been reported to have a role in controlling the development and the functions of rheumatoid-associated cells including miR-16, -132, -146, and -155 [34]. The two most studied miRNAs in patients with RA are miR-146 and miR-155, which play a role in the development of innate and adaptive immune cells, are essential for the maintenance of immune homeostasis and are up-regulated under inflammatory conditions [34, 35]. They represent biomarkers for RA activity that might be useful for treatment follow-up, because the above miRNAs' plasma levels inversely correlated with parameters of disease activity (DAS-28, VAS, number of tender joints [34]). Despite the fact that a few miRNAs have been found to contribute to different aspects of RA pathogenesis and have a high therapeutic potential, unique signatures of miRNAs in RA have not yet been found. However, the potential of miRNAs in the regulation of various immune pathways and as mediators of interactions between cells makes them ideal drug targets. That is why the search for specific expression signatures of miRNAs in patients with RA for prognostic/diagnostic purposes has become clinically realistic [34].

Therefore, the potential to therapeutically regulate the level of miRNA may provide new possibilities for optionally regulating the immune system, and preventing or attenuating disease progression. miRNA-based therapies have some advantages over current drug strategies: they are a class of highly specific and effective regulators, and one miRNA may regulate several genes at the same time, leading to effects on multiple signalling pathways [35]. However, before we can envisage the development of miRNA-based therapies for the treatment of RA, a deeper knowledge of the functions of already and/or newly identified miRNAs is needed.

Genetic test available for rheumatoid arthritis

Every day, millions of RA patients take drugs that will not help them. Early diagnosis of rheumatoid arthritis is significant in so much as the primary treatment can be started better. Pharmacogenetic and pharmacogenomic studies, which help determine the genetic profile of individual patients, may bring us closer to personalized medicine. Therefore, discovery of specific biomarkers for RA diagnosis and treatment is still a dream of rheumatologists. Unfortunately, at the present day pharmacogenetic/pharmacogenomic tests are not common knowledge. This can be due to several reasons such as heterogeneity of RA, incomplete information about pathogenesis of the disease, small sample size as well as other non-genetic factors (demographic, environmental and clinical or serological markers) that can influence or predict the efficacy or toxicity of a drug in patients with RA. Moreover, there are many reasons why it is important to identify the specific genes and epigenetics changes involved in rheumatoid arthritis development, severity and response to treatment. These reasons include predicting who will develop RA, predicting how severe the disease will be, predicting which treatment someone with RA will re-

spond to and identifying new targets for treatment. In the coming years, the major challenges for researchers will be to describe how genetic/epigenetic variations affect the molecular function in specific cell subtypes and are connected with the susceptibility to and severity of RA. Developing evaluation tools that will benefit from individual genomic/epigenetic information may have a higher predictive value, enabling the translation of the latest results of genetic testing into clinical practice [18]. Recent advances in new technologies, such as NGS, should allow a more personalized approach to clinical care, with enhanced risk stratification and treatment choices, based on information from the individual genetic/epigenetic background [18]. Although the pharmacogenetic/pharmacogenomic methods may be expensive, the high cost of biologic treatment in patients with RA increases the likelihood that companion diagnostics may be cost effective. Moreover, identification of the genetic/epigenetic factors underlying the variability in drug treatment responses may lead to early detection of responder and/or non-responder patients, and thus will promote development of better and more effective therapeutic strategies for patients with rheumatoid arthritis.

Personalized medicine in the future

The sooner the right diagnosis of rheumatoid arthritis is made, the smaller is the risk of disease progression to pathology. Further studies on RA should allow for the identification of disease-specific genes at the stage when their tolerance by the organism is still preserved (before auto-aggression develops). The objective of future studies should be to explore mechanisms of breaching of tolerance in spatiotemporal *in vivo* systems with the additional advantage of rationalising the use of existing therapeutics, i.e. administering the right drug at the right time and place to the right person [37].

One reason why personalized medicine is so important for patients with RA is the fact that about one-third of patients do not respond to a specific biological therapy. The course of RA is highly multifaceted. This probably explains different responses to treatment in each patient [38]. Current studies carried out in the group of patients with RA do not take into account separate pathotypes responsible for treatment response. The results of British studies indicate a strong correlation between high level of disability in the group of patients with RA and weak response to TNF inhibitors [39].

The lack of therapy with non-steroid anti-inflammatory drugs or MTX at the same time leads to decreased probability of response, in particular to etanercept. Lower probability of remission is also observed in the group of women. The results of Swedish studies indicate better results in TNF inhibition in the group of patients with a lower level of disability who are treated with DMARDs. However, the outcomes of the Danish studies show a weaker response to first treatment of anti-TNF in the group of older patients as well as patients treated with prednisolone [39].

In future, a 'composite scoring system' based on a number of biomarkers and demographic factors should constitute the basis of personalized medicine in rheumatoid arthritis. As a result, the symptoms could be used to tailor the most effective treatment for each patient. This will limit adverse effects, improve outcomes and reduce costs. "Head-to-head" studies relating to different biological therapies that could indicate appropriate therapy selection are increasingly taken into consideration. By way of example, there are outcomes of studies indicating that tocilizumab could be more appropriate as the first choice in biological therapy of patients who do not tolerate MTX. However, these studies must be interpreted in the context of the precisely selected clinical trial population [38].

New criteria have been introduced to the process of rheumatoid arthritis diagnosis which take into account the use of anti-citrullinated protein antibodies (ACPA). Identification of markers used for distinguishing undifferentiated arthritis from RA is the next step. Identification of biomarkers at this stage as well as the development of tools combining markers and stage-related clinical characteristics will impact on treatment initiation, its selection and duration [40]. In order to induce remission, and thus to prevent irreversible damage to the joints in rheumatoid arthritis, early diagnosis and timely treatment initiation are of great significance. Early diagnosis should be preferably carried out in the pre-clinical/asymptomatic phase. Several studies have documented the occurrence of ACPA as well as rheumatoid factor (RF) before the development of RA [41].

Tests for measuring known diagnostic biomarkers are commonly used in clinical practice. It is estimated that 70% of therapeutic decisions made by physicians are based on the results of these tests. However, the implementation of novel biomarkers into clinical practice has proved to be a long and difficult process that includes convincing physicians. The assessment of the impact of using the biomarker on general health constitutes an important step to guarantee the uptake of the biomarker in clinical practice and further optimization of its use. This research area is increasingly important, as biomarkers are introduced more often to clinical practice. Due to the complexity and heterogeneous nature of rheumatoid arthritis, it is unlikely that a single cytokine may provide sufficient discrimination. Currently there are many reliable cytokine assays available with multiplex formats that are leading in this area (although in the case of RA it may not be an appropriate solution

due to RF interferences). Tests have proven to be clinically useful in the case of other diseases; therefore their implementation in rheumatology should be easy (technically). It is however necessary to establish the exact performance characterization and quality assurance for the specific cytokines of interest in RA. At present, the complexity of the disease that is related to cytokine networks constitutes a limitation in RA. In future, multiple biomarker signatures that are based on genetics as well as proteomic markers may represent a more realistic approach towards personalized medicine in RA. Such multifactorial analysis may potentially reveal patterns rather than individual biomarkers. A single IL-7 is able to predict diagnosis at a very early stage of the disease, whereas a more complex combination of markers may be needed to predict the response to therapy and define subsets of patients with more advanced disease [42].

Summary

There is a great need for reliable biomarkers relating to the response to biological treatment in order to improve responsiveness, preserve the structure and functions of the joints as well as to reduce the costs of treatment. To date, the results of some tests have confirmed that the treatment response to rituximab can be predicted due to numerous clinical characteristics relating to the response to TNF inhibition as well as the presence of antibodies in the serum. Current response indicators can predict the probability of response to a drug or the quality of the response, but a lack of response cannot be predicted. New ACR/EULAR diagnostic criteria for rheumatoid arthritis attach considerable importance to antibodies. It cannot be excluded that in future RA cohorts will also include seropositive patients. So far it seems reasonable that patients with seronegative RA are treated with another medicine before introducing rituximab [39, 43].

Before molecular disease diagnosis, as a basis of the individualized approach, becomes standard in the future, personalized medicine will need to face some fundamental issues. The first, key issue is the necessity to identify genetic variation through testing a million single nucleotide polymorphisms (SNP) that occur in the genome. Then, it should be indicated which SNPs are responsible for the disease and could constitute clinically usable markers. Financial matters would be another issue. The above-mentioned search for disease markers is possible only using expensive genotyping methods and detailed understanding of biological defence mechanisms. Another issue is limited access to appropriate tissues in the case of multiple diseases, which makes it difficult to type protein markers. Both proteomic and computing technologies need further improvements in order to be used effectively for the analysis of this type of data.

There is a need to search for reliable biomarkers, e.g. genetic markers, to predict rare adverse events. These markers should have detection ability adjusted to small samples. There is however a precedent for this, 18 for example, in liver toxicity from flucloxacillin and HLA-DRB*5701 (OR > 80), or thiopurine S-methyltransferase gene polymorphism and azathioprine-induced bone marrow suppression [40].

In order to make the best possible use of personalized therapies, biomarkers must be identified and validated. Therefore, there is a need to develop new regulations that define the interaction between industry and academia in terms of regulatory control.

There is also a need to develop new standards for stakeholders participating at all levels of personalized medicine implementation, from biomarkers' validation to informed consent of the patient. The implementation of personalized medicine is limited for several reasons. The basic example is the lack of stakeholders' involvement and defined standards of conduct as well as inappropriate funding policy at the European level and the access to data. The healthcare system constitutes another barrier to the implementation of personalized medicine. These challenges can and should be effectively addressed. In order for personalized medicine to be effectively practised, systematic actions must be urgently taken to remove barriers to its implementation [44].

The authors declare no conflict of interest.

References

1. Chmara E. Medycyna spersonalizowana. Farmacja Współczesna. 2011; 4: 133-135.
2. Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. Trends Mol Med 2001; 7: 201-204.
3. Ginsburg GS, McCarthy JJ. Personalized medicine: revolutionizing drug Discovery and patient care. Trends in Biotechnology 2001; 19: 491-496.
4. Hamburg MA, Collins FS. The path to Personalized Medicine. N Engl J Med 2010; 10: 1-4.
5. Ruano G. Quo Vadis personalized medicine? Personalized Med 2004; 1: 1-7.
6. Gremese E, Salaffi F, Bosselo SA, et al. Very early rheumatoid arthritis as a predictor of remission: a multicentre real life prospective study. Ann Rheum Dis 2013; 72: 858-862.
7. Smolen JS, Landewé R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Ann Rheum Dis 2014; 73: 492-509.
8. Burmaster G, Lanas A, Biasucci L, et al. The appropriate use of non-steroidal anti-inflammatory drugs In rheumatic disease: opinions of multidisciplinary European expert panel. Ann Rheum Dis 2011; 70: 818-822.

9. Smolen JS, Landewé R, Breedveld FC, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. Ann Rheum Dis 2010; 69: 631-637.
10. Aviña-Zubieta JA, Abrahamowicz M, De Vera MA, et al. Immediate and past cumulative effects of oral glucocorticoids on the risk of acute myocardial infarction in rheumatoid arthritis: a population-based study. Rheumatology 2013; 52: 68-75.
11. Whittle SL, Colebatch AN, Buchbinder R, et al. Multinational evidence-based recommendations for pain management by pharmacotherapy in inflammatory arthritis: integrating systematic literature research and expert opinion of a broad panel of rheumatologists in the 3e Initiative. Rheumatology 2012; 51: 1416-1425.
12. Salliot C, Van der Heijde D. Long term safety of Methotrexate monotherapy in Rheumatoid Arthritis patients: A Systematic Literature Research. Ann Rheum Dis 2009; 68: 1100-1104.
13. Visser K, Katchamart W, Loza E, et al. Multinational evidence-based recommendations for the use of methotrexate In rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists In the 3E. Ann Rheum Dis 2009; 68: 1086-1093.
14. Szekanecz Z, Mesko B, Poliska SZ, et el. Pharmacogenetics and pharmacogenomics in rheumatology. Immunol Res 2013; 56: 325-333.
15. Kooloos WM, Huizinga TWJ, Guchelaar HJ, et al. Pharmacogenetics in Treatment of Rheumatoid Arthritis. Current Pharmaceutical Design 2010; 16: 164-175.
16. Giannopoulou EG, Elemento O, Ivashkiv LB. Use of RNA sequencing to evaluate rheumatic disease patients. Arthritis Res Ther 2015; 17: 167.
17. Mohan C, Assassi S. Biomarkers in rheumatic diseases: how can they facilitate diagnosis and assessment of disease activity? BMJ 2015; 351: h5079.
18. Goulielmos GN, Zervou MI, Myrthianou E, et al. Genetic data: The new challenge of personalized medicine, insights for rheumatoid arthritis patients. Gene 2016; 583: 90-101.
19. Zheng W, Rao S. Knowledge-based analysis of genetic associations of rheumatoid arthritis to inform studies searching for pleiotropic genes: a literature review and network analysis. Arthritis Res Ther 2015; 17: 202.
20. Maranville JC, Di Rienzo A. Combining genetic and nongenetic biomarkers to realize the promise of pharmacogenomics for inflammatory diseases. Pharmacogenomics 2014; 15: 1931-1940.
21. Malik F, Ranganathan P. Methotrexate pharmacogenetics in rheumatoid arthritis: a status report. Pharmacogenomics 2013; 14: 305-314.
22. Gervasini G. Polymorphisms in methotrexate pathways: what is clinically relevant, what is not, and what is promising. Curr Drug Metab 2009; 10: 547-566.
23. Zhu H, Deng FY, Mo XB, et al. Pharmacogenetics and pharmacogenomics for rheumatoid arthritis responsiveness to methotrexate treatment: the 2013 update. Pharmacogenomics 2014; 15: 551-566.
24. Xie X, Zhang D, Chen JW, et al. Pharmacogenomics of biological treatment in rheumatoid arthritis. Expert Opin Biol Ther 2014; 14: 157-164.
25. Breedveld F. TNF antagonists opened the way to personalized medicine in rheumatoid arthritis. Mol Med 2014; 20: 7-9.
26. Burska AN, Roget K, Blits M, et al. Gene expression analysis in RA: towards personalized medicine. Pharmacogenomics J 2014; 14: 93-106.
27. Naranbhai V, Fairfax BP, Makino S, et al. Genomic modulators of gene expression in human neutrophils. Nat Commun 2015; 6: 7545.
28. Walsh AM, Whitaker JW, Huang CC. Integrative genomic deconvolution of rheumatoid arthritis GWAS loci into gene and cell type associations. Genome Biol 2016; 17: 79.
29. Zhu Z, Zhang F, Hu H, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet 2016; 48: 481-487.
30. Kim TH, Choi SJ, Lee YH, et al. Gene expression profile predicting the response to anti-TNF treatment in patients with rheumatoid arthritis; analysis of GEO datasets. Joint Bone Spine 2014; 81: 325-330.
31. Sanayama Y, Ikeda K, Saito Y, et al. Prediction of therapeutic responses to tocilizumab in patients with rheumatoid arthritis: biomarkers identified by analysis of gene expression in peripheral blood mononuclear cells using genome-wide DNA microarray. Arthritis Rheum 2014; 66: 1421-1431.
32. Hurd PJ, Nelson CJ. Advantages of next-generation sequencing versus the microarray in epigenetic research. Brief Funct Genomic Proteomic 2009; 8: 174-183.
33. Castro-Villegas C, Pérez-Sánchez C, Escudero A, et al. Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNF-α. Arthritis Res Ther 2015; 17: 49.
34. Duroux-Richard I, Jorgensen C, Apparailly F. What do microRNAs mean for rheumatoid arthritis? Arthritis Rheum 2012; 64: 11-20.
35. Vicente R, Noël D, Pers YM, et al. Deregulation and therapeutic potential of microRNAs in arthritic diseases. Nat Rev Rheumatol 2016; 12: 211-220.
36. Churov AV, Oleinik EK, Knip M. MicroRNAs in rheumatoid arthritis: altered expression and diagnostic potential. Autoimmun Rev 2015; 14: 1029-1037.
37. Benson RA, Patakas A, McQueenie R, et al. Arthritis in space and time – to boldly go! FEBS Letters 2011; 585: 3640-3648.
38. Richardson S, Isaacs J. Novel immunotherapies for rheumatoid arthritis. Clin Med 2013; 13: 391-394.
39. Isaacs JD, Ferraccioli G. The need for personalised medicine for rheumatoid arthritis. Ann Rheum Dis 2011; 70: 4-7.
40. Miossec P, Verweij CL, Klareskog L, et al. Biomarkers and personalised medicine in rheumatoid arthritis: a proposal for interactions between academia, industry and regulatory bodies. Ann Rheum Dis 2011; 70: 1713-1718.
41. Verweij CL. Transcript profiling towards personalized medicine in rheumatoid arthritis. Neth J Med 2009; 67: 364-371.
42. Burska A, Boissinot M, Ponchel F. Cytokines as biomarkers in rheumatoid arthritis. Mediators Inflamm 2014; 2014: 545493.
43. Smolen JS, Aletaha D. Forget personalised medicine and focus on abating disease activity. Ann Rheum Dis 2013; 72: 3-6.
44. Horgan D, Jansen M, Leyens L, et al. An index of barriers for the implementation of personalised medicine and pharmacogenomics in Europe. Public Health Genomics 2014; 17: 287-298.