REVIEW ARTICLE

Assessing Immunogenicity of Biologic Drugs in Inflammatory Joint Diseases: Progress Towards Personalized Medicine

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Abstract

Biologic drugs have greatly improved treatment outcomes of inflammatory joint diseases, but a substantial proportion of patients either do not respond to treatment or lose response over time. Drug immunogenicity, manifested as the formation of anti-drug antibodies (ADAb), constitute a significant clinical problem. Anti-drug antibodies influence the pharmacokinetics of the drug, are associated with reduced clinical efficacy, and an increased risk of adverse events such as infusion reactions. The prevalence of ADAb differs among drugs and diseases, and the detection of ADAb also depends on the assay format. Most data exist for the tumor necrosis factor-alpha inhibitors infliximab and adalimumab, with a frequency of ADAb that ranges from 10 to 60% across studies. Measurement of ADAb and serum drug concentrations, therapeutic drug monitoring, has been suggested as a strategy to optimize therapy with biologic drugs. Although the recent randomized clinical Norwegian Drug Monitoring (NOR-DRUM) trials show promise towards a personalized medicine prescribing approach by therapeutic drug monitoring, several challenges remain. A plethora of assay formats, with widely differing properties, is currently used for measuring ADAb. Comparing results between different assays and laboratories is difficult, which complicates the development of cut-offs necessary for guidelines and the implementation of ADAb measurements in clinical practice. With the possible exception of infliximab, limited data on clinical relevance and cost effectiveness exist to support therapeutic drug monitoring as a routine clinical strategy to monitor biologic drugs in inflammatory joint diseases. The aim of this review is to provide an overview of the characteristics and prevalence of ADAb, predisposing factors to ADAb formation, commonly used assessment methods, clinical consequences of ADAb, and the potential implications of ADAb assessments for everyday treatment of inflammatory joint diseases.

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Key Points

In treatment with biologic drugs, anti-drug antibody formation is associated with reduced clinical efficacy, and an increased risk of adverse events such as infusion reactions.

Therapeutic drug monitoring of biologic drugs has shown promise as a strategy for treatment optimization, supported by emerging evidence including the recent randomized Norwegian Drug Monitoring (NOR-DRUM) clinical trials, but more data are needed.

Widely differing characteristics of anti-drug antibody assays, a lack of reliable markers to identify patients at risk of anti-drug antibody development, and limited guidance on the interpretation of results are barriers that must be overcome to harness the potential of therapeutic drug monitoring in clinical practice.

1 Introduction

Biologic drugs have become a cornerstone of treatment in inflammatory joint diseases (IJDs), including rheumatoid arthritis (RA), spondyloarthritis (SpA), and psoriatic arthritis (PsA), and have greatly improved treatment outcomes. Biologic drugs used in the treatment of IJDs include tumor necrosis factor inhibitor (TNFi) monoclonal antibodies (mAbs) and receptor fusion protein, a T-cell co-stimulation modulator fusion protein, an anti-CD20 mAb, and anti-interleukin (IL)-17A, anti-IL-6 receptor, and anti-IL-12/23 mAbs. Despite the advances in therapy, a substantial proportion of patients either do not respond to treatment or lose response over time [1–3].

Drug immunogenicity, manifested as the formation of anti-drug antibodies (ADAb), is a major cause of non-response [4, 5]. All biologic drugs, being large and complex allogenic proteins, are able to elicit patient immune responses against the drug, with the production of ADAb that influence the effectiveness and pharmacokinetics of the drug, namely by blocking binding to its target and by accelerating clearance of the drug [6]. Anti-drug antibody formation is a significant clinical problem leading to reduced clinical efficacy, and an increased risk of adverse events such as infusion reactions [4, 5].

Measurement of ADAb and serum drug concentrations, TDM, has been suggested as a strategy to optimize treatment with biologic drugs [7–9]. While TDM has shown promise and may be an effective strategy in personalizing treatment decisions in IJDs, there are also several challenges. While different methods used for drug measurements usually give comparable results, and published data on therapeutic drug concentrations may be relevant for laboratories and clinicians worldwide, this is not the case for ADAb [10, 11]. A plethora of assay formats, with widely differing properties, is currently used for measuring ADAb [12, 13]. Comparing results between different assays and laboratories is difficult, which complicates the implementation of ADAb measurements in clinical practice. The recent "points to consider (PtC)" from the European Alliance of Associations for Rheumatology (EULAR) Task Force on TDM of biologic drugs address the clinical relevance of ADAb assessments, and highlight several knowledge gaps within this field [10].

The aim of this review is to provide an overview of the characteristics and prevalence of ADAb, predisposing factors to ADAb formation, commonly used assessment methods, clinical consequences of ADAb, and the potential implications of ADAb assessments for everyday treatment of IJDs.

2 Structure and Evolution of Biologic Drugs

The first therapeutic mAb used in humans, the murine mAb muromonab (OKT3), was approved in 1986 for the treatment of transplant rejections. This and other early therapeutic mAbs were fully murine and rapidly induced anti-murine immune responses in the patients [14]. They were thus unsuitable for repeated use in diseases such as IJDs. To overcome these problems, chimeric mAbs were introduced in the 1990s. With recombinant antibodies, highly immunogenic murine sequences were replaced with human sequences, and only variable regions of light and heavy chains remained murine [15]. Following advancements in antibody engineering, humanized antibodies became available in the late 1990s. A humanized antibody is an immunoglobulin where only the complementarity-determining regions, the variable part of the antibody that determines the specific antigen recognition site, are non-human [15]. Adalimumab was the world's first fully human therapeutic antibody and was approved for the treatment of RA in 2002 [15]. Although fully human antibodies are presumably less immunogenic than chimeric and humanized antibodies, they still contain allogenic sequences within the complementarity determining region [16].

3 Characteristics of ADAb and Mechanisms of Formation

The exact immunological mechanisms behind immune responses directed against therapeutic mAbs are not completely understood [17, 18], but the main mechanism for development of ADAb is thought to be T cell dependent with peptides from the therapeutic mAb presented by the human leukocyte antigen (HLA). Identifying and removing T-cell epitopes from a putative therapeutic molecule has been shown to be important to lower immunogenicity in the development of new biologic drugs [18]. Additionally, ADAb formation may be triggered by T-cell independent activation of B cells by cross-linking of B-cell receptors [17]. This mechanism depends on multiple closely spaced epitopes, which may be attributed to impurities and aggregates in the drug formulation [19].

A number of studies have shown that the risk of ADAb formation is greatest early in the treatment course [20–24], corresponding to the immune system's reaction to an antigen. As in antibody formation generally, the response is first IgM, maturing into IgG. IgG_1 and IgG_4 are the dominating isotypes and IgG_4 has been suggested to develop during long-term treatment [25, 26].

The ADAb formed may be neutralizing—they block the antigen-binding capacity of the therapeutic molecule—or

non-neutralizing [6]. Neutralizing ADAb, i.e., anti-idiotype ADAb binding to the antigen-binding site will directly affect the ability of the therapeutic antibody to bind its target and diminish the efficacy of the drug. Non-neutralizing ADAb, i.e., anti-allotype ADAb binding to epitopes outside the antigen-binding region, may not directly interfere with antigen binding, but could form complexes with drugs and theoretically enhance Fcγ receptor-mediated drug clearance [27]. Both neutralizing and non-neutralizing ADAb could also be associated with immune complex-mediated alterations in Fc-mediated effector functions, including enhanced complement activation and Fcγ receptor-mediated effects of the drug [28]. Neutralizing ADAb are presumably of greatest clinical relevance, but both types of ADAb may decrease the availability of the drug, lessen its clinical effect, and affect the risk of adverse events.

4 Prevalence of ADAb in IJDs

An overview of the prevalence of ADAb formation to biologic drugs in IJDs is shown in Table 1. The most robust data exist for the TNFi infliximab and adalimumab with ADAb formation reported in 10–60% of patients across diseases [4, 5, 29]. For the fully human TNFi golimumab, the prevalence of ADAb is lower, which ranges from 2 to 15% across studies. Because of its structure, etanercept is presumably less immunogenic than other TNFi. Most studies

report no formation of anti-etanercept antibodies [30–33], but anti-etanercept antibodies have been reported in up to 13% in RA [34–37]. Anti-drug antibodies measured in these studies were non-neutralizing, and not associated with lower drug concentrations or a lack of response. For certolizumab pegol, the reported frequency of ADAb in IJDs ranges from 5 to 65% [38–44]. The large variation is notable, but may be attributed to different time points, assay formats (including differences in detection of anti-PEG antibodies), and study populations.

Data regarding prevalence of ADAb are scarce for non-TNFi (Table 1). The discrepancy in available data for non-TNFi versus TNFi is likely related to differences in the duration of marketing and the more extensive use of TNFi.

The differences in the prevalence of ADAb development between drugs, both within and between classes, are striking. Inherent differences in the pharmacological compound as well as their mechanisms of action are important factors explaining the differences, as further discussed in the next section. Advances in antibody engineering and production likely explain lower immunogenicity of newer biologic drugs. However, comparisons of immunogenic potential across biologic drugs and diagnoses should be done with caution. The number of studies and study populations, the indications for use, as well as dosing regimens and use of concomitant immunomodulators differ among drugs and diagnoses. Importantly, the use of different assays for ADAb

Table 1 Prevalence of anti-drug antibody formation to biologic drugs in inflammatory joint diseases

Biologic drug	Target protein	Molecular structure	Across IMIDs	RA	PsA	SpA	References
TNF inhibitors							
Infliximab	TNFα	Chimeric IgG$_1$κ mAb	25% (95% CI 20–32)	8–62%	15–33%	6–69%	[4, 5]
Adalimumab	TNFα	Fully human IgG$_1$κ mAb	14% (95% CI 9–22) 25% (range 0–87%)	0–51%	0–54%	8–39%	[4, 5, 29]
Golimumab	TNFα	Fully human IgG$_1$κ mAb	2–15 %	2–10%	6%	0–6%	[5, 89, 114, 117, 167]
Etanercept	TNFα (lymphotoxin)	TNF receptor 2-IgG$_1$ Fc fusion protein	–	0–13%	0%	0%	[5, 33–37]
Certolizumab pegol	TNFα	Pegylated Fab fragment of humanized IgG$_1$κ mAb	6%	5–65%	–	–	[38–44]
Non-TNF inhibitor biologic drugs							
Tocilizumab	IL-6 receptor	Humanized IgG$_1$κ mAb	n/a	0–2%	n/a	n/a	[59, 168–170]
Abatacept	CD80/CD86 (T cell co-stimulator proteins)	CTLA-4-IgG$_1$ Fc fusion protein	n/a	1–7%	n/a	n/a	[60–62, 171, 172]
Rituximab	CD20	Chimeric IgG$_1$κ mAb	n/a	9–22%	n/a	n/a	[173–175]
Sekukinumab	IL-17A	Fully human IgG$_1$κ mAb	n/a	n/a	< 1%	< 1%	[176]
Ixekizumab	IL-17A	Humanized IgG$_4$ mAb	n/a	n/a	11–16%	–	[177]
Ustekinumab	IL-12/23	Fully human IgG$_1$κ mAb	n/a	n/a	8–11%	n/a	[5]

CI confidence interval, *CTLA-4* cytotoxic T lymphocyte-associated protein-4, *IL* interleukin, *IMIDs* immune-mediated inflammatory diseases, *mAb* monoclonal antibody, *n/a* - not applicable, *PsA* psoriatic arthritis, *RA* rheumatoid arthritis, *SpA* spondyloarthritis, *TNFα* tumor necrosis factor alpha

detection undoubtedly influences the results [10, 11] (as discussed in detail below).

5 Factors Affecting ADAb Development

The formation of ADAb to TNFi and other biologic drugs is a complex process, where both treatment-related and patient-related factors contribute. Identification of factors associated with ADAb development is important as they may aid in the selection of treatment and identify patients who could benefit from co-medication and close monitoring [45].

5.1 Treatment-Related Factors

5.1.1 Drug-Related Factors

As described above, all biologic drugs are allogenic molecules that may elicit immune responses, with molecules containing non-human regions more likely to trigger immune response [16]. Drug aggregates and impurities may also increase immunogenicity. The pharmacodynamic properties of individual drugs presumably also influence immunogenicity, i.e., drugs that inhibit B cells or IL-6 pathways likely have lower immunogenic potential [5].

5.1.2 Concomitant Immunosuppression

Several studies have found methotrexate comedication to reduce the risk of ADAb to adalimumab [46–51] and infliximab [50–52] therapy in patients with RA. The protective effect has been proposed to be dose dependent [48, 49]. Although concomitant use of methotrexate is less common in patients with SpA, methotrexate has also been suggested to reduce the risk of ADAb in patients with SpA receiving adalimumab and infliximab therapy [45, 53]. Data for sulfasalazine and leflunomide are limited [54]. In two studies comparing the effect of concomitant methotrexate to other concomitant synthetic disease-modifying antirheumatic drugs, patients takig methotrexate had a higher probability of detectable TNFi serum concentrations and clinical response [55, 56]. Unfortunately, results for ADAb were not reported.

5.1.3 Route and Mode of Administration

Subcutaneous (SC) drug administration is commonly perceived to be associated with enhanced immunogenic potential, as compared with intravenous (IV) administration. This has been proposed to be due to efficient antigen presentation in the immune response to subcutaneous proteins [57]. While enhanced immunogenic potential has been demonstrated for some SC biologic drugs, the prevalence of ADAb

formation is similar for SC and IV formulations for other biologics [57, 58]. The latter has been suggested for tocilizumab and abatacept, which are used in both SC and IV formulations in IJDs [59–62]. Low drug doses, episodic therapy, and drug holidays are associated with an increased risk of ADAb to TNFi [5, 45]. This increased risk is likely attributed to low drug concentrations, which has been linked to ADAb formation [45, 63–65].

5.2 Patient-Related Factors

5.2.1 Genetic Factors

Because of the known involvement of antigen-presenting cells and HLA class II molecules in the mechanism underlying ADAb formation described above, variations in HLA class II genes have been suspected to be involved in ADAb formation [66]. This is supported by two genome-wide association studies that have linked the HLA-DQA*05 allele group to ADAb formation to biologic drugs in patients with IJDs other than immune-mediated inflammatory diseases [67, 68]. A study of patients with RA and hidradenitis suppurativa receiving adalimumab therapy examining the HLA-DQB1 and HLA-DRB1 loci found allele groups associated with both an increased and a reduced risk of ADAb [69]. The haplotype DRB1*03:01~DQB1*02:01~DQA1*05:01, which is strongly associated with several autoimmune diseases [70, 71], has also been associated with an increased risk of ADAb in patients with Crohn's disease treated with infliximab [72]. A similar association was also found in a recent sub-analysis of the Norwegian Drug Monitoring (NOR-DRUM) trials assessing patients with immune-mediated inflammatory diseases receiving infliximab therapy [73]. This study, however, found the HLA-DQ2 haplotypes DQB1*02:01~DQA1*05:01 and DQB1*02:02~DQA1*02:01 to be the most important HLA variants associated with an increased risk of ADAb. HLA-DQB1 and HLA-DQA1 genes together form the HLA-DQ2 molecule, which was shown to bind strongly to peptide sequences derived from infliximab. The fact that similar findings have been presented by studies including different diseases suggests that the molecular mechanisms underlying ADAb formation are not disease specific. Other genetic polymorphisms, such as in the CXCL12, IL-10, and Fc gamma receptor (FCGR3A) genes, have also been proposed to be associated with a risk of ADAb formation to TNFi [67, 74–76]. Whilst an area of research interest, at present, there is no definitive evidence to support genetic testing to predict ADAb formation as most studies are limited by small patient numbers or a lack of replication.

5.2.2 Disease Type

In the NOR-DRUM trials, which included patients with RA, PsA, SpA, and inflammatory bowel disease, significant differences in immunogenicity were found between diagnoses, with patients with RA having the highest rate of ADAb formation and patients with SpA the lowest [45]. Despite concomitant use of immunosuppressant drugs being more common in patients with RA [77, 78], lower rates of ADAb formation in patients with SpA compared with RA have also been reported previously [5, 50]. Infliximab-treated patients with RA receive a lower starting dose than the other diagnoses, which might contribute to a higher proportion of patients with ADAb in this group. Another explanation may be related to higher levels of B-cell activating factor in patients with RA. This cytokine may be involved in the pathogenesis of RA, and has also been associated with ADAb to TNFi [79, 80].

5.2.3 Other Patient-Related and Disease-Related Factors

Smoking [45, 64, 67, 81], higher disease activity [20, 45, 81, 82], and longer disease duration [20] are other patient-related factors associated with ADAb to TNFi. Higher body mass index has been linked to the immunogenicity of TNFi in patients with Crohn's disease [64]. Whether this relates to lower drug concentrations needs further clarification. Furthermore, anti-nuclear antibodies (ANA), rheumatoid factor positivity, and female sex have also been associated with ADAb to infliximab in patients with RA [83, 84].

5.2.4 Methods for Assessment of ADAb

Various assay formats exist for the measurement of ADAb [12, 13]. Assays may have widely differing capture and detection principles and some assays include elaborate sample pre-treatment steps. As a consequence, test results from different assays are rarely comparable, and clinicians should be familiar with the properties of the assay provided by their laboratory [10, 11]. Perhaps the most important factor to consider is whether the assay specifically measures neutralizing antibodies or also measures non-neutralizing antibodies (as discussed below). Most ADAb assays are *drug sensitive*, i.e., they have limited ability to detect ADAb in the presence of a circulating drug. *Drug-tolerant* assays usually incorporate sample pre-treatment steps and may allow the detection of ADAb in the presence of a drug [85]. There are three main categories of assay formats for the measurement of ADAb; immunoassay formats, liquid-phase methods, and functional assays.

5.3 Immunoassay Formats

Immunoassay formats are widely used in clinical practice and research, as they are (comparably) low-cost and high-throughput methods. The formats include *antibody capture tests*, *bridging assays, antigen binding tests,* and *inhibition assays*, as illustrated in Fig. 1a–d. The terminology may be confusing and (mis)classification of assay formats based on detection principles is common. In the subsequent section, we present an overview of the most common immunoassays used in routine practice. In most immunoassay formats, the tracer molecule can be conjugated to optional detection labels, commonly an enzyme, a radioisotope, or a fluorescent/electrochemiluminescent molecule. When an enzyme-substrate reaction is used as the detection method, the assay is commonly referred to as an enzyme-linked immunosorbent assay or enzyme immunoassay, irrespective of the assay format or assay property. This is common with other detection methods as well, i.e., radioimmunoassa or immunoradiometric assay with radioactive tracers, fluoroimmunassay or immunofluorometric assay with fluorescent labels, and electrochemiluminescence immunoassay with electrochemiluminescent labels. Although these names and acronyms are well established, they are not always used correctly and rarely reveal important information about an assay's property. Below (and in Fig. 1a–d), we briefly describe common immunoassays used for ADAb detection based on format, not the detection label.

In *antibody capture tests*, ADAb is captured by binding to the solid-phase bound biologic drug, and quantified by labeled anti-human antibody tracers (Fig. 1a). These assays are inexpensive and easy to use, but limitations include high background signals and the potential for false-positive results due to unspecific binding. *Bridging assays* (Fig. 1b) are widely used to detect ADAb, partly because they can be established using the biologic drug both as a solid-phase and tracer protein. However, as they rely on both ADAb Fab' fragments to be free to cross-link the biologic drug molecules [12], they are highly drug sensitive (i.e., have limited ability to detect ADAb in the presence of a detectable drug) and also have limited ability to detect ADAb of IgG$_4$ subclass [86, 87]. Hence, bridging assays risk underestimating ADAb. In addition, if an unmodified (whole IgG) biologic drug is used as a solid-phase and tracer protein, interference from (Fc-binding) rheumatoid factor is a risk particularly relevant in patients with RA. In an *antigen-binding test*, beads coated with protein A or anti-human Fc antibodies capture immunoglobulins in the sample (Fig. 1c). Anti-drug antibodies immobilize to the beads then bind to labeled fragments of the biologic drug [12]. *Inhibition assays* are based on the ability of ADAb to inhibit binding of the labeled biologic drug to its target molecule (e.g., TNFα) [Fig. 1d], and thus selectively measure neutralizing antibodies [88, 89].
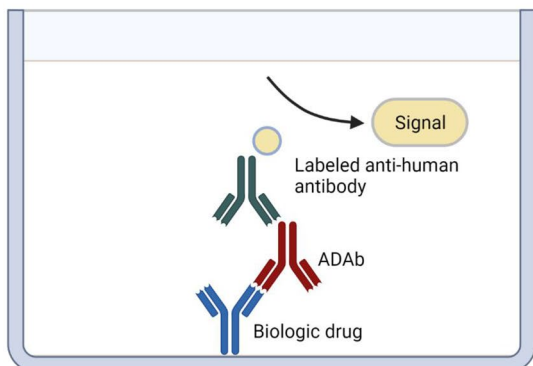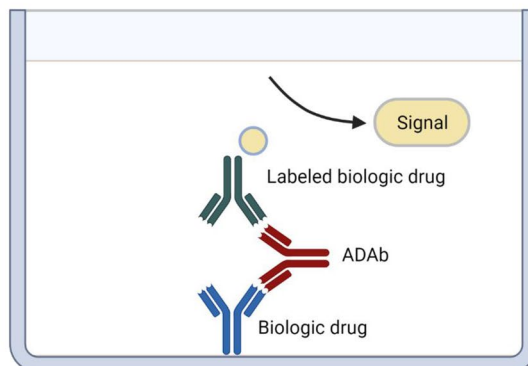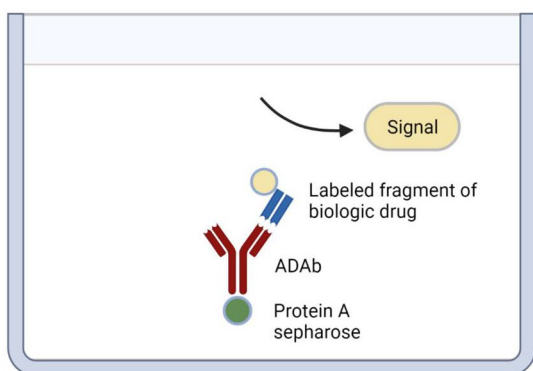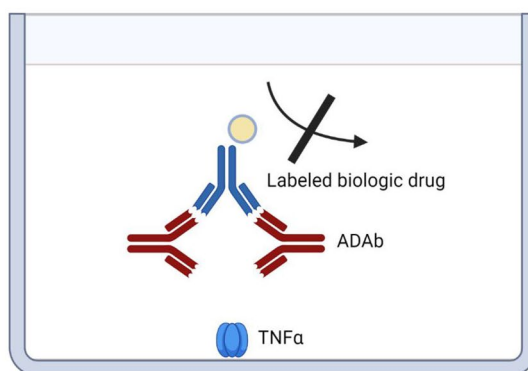
a. Antibody capture test

b. Bridging test

c. Antigen binding test

d. Inhibition test

Fig. 1 Immunoassay formats used for anti-drug antibody (ADAb) detection. **a** In antibody capture tests, ADAb is "captured" by binding to a solid-phase bound biologic drug, and quantified by labeled anti-human antibody tracers. **b** In the bridging test, ADAb are detected by cross-linking of the solid-phase and labeled biologic drug molecules. **c** In the antigen-binding test, human immunoglobulins are captured by protein A (or anti-human antibody) coated sepharose beads. After non-bound serum components are washed off, ADAb are detected by binding to labeled biologic drug/fragment. **d** In the inhibition test, samples are preincubated with a known amount of labeled biologic drug. Anti-drug antibodies inhibit binding of a labeled biologic drug to its target molecule (e.g., tumor necrosis factor alpha [TNFα]). The signal is thus inversely proportional to the amount of ADAb in the sample. Assay antibodies are illustrated as intact immunoglobulins, but immunoglobulin fragments are preferred in most formats. Created with BioRender

While inhibition assays and most antigen-binding tests (including Fab-fragments) measure neutralizing ADAb, antibody capture tests and bridging tests (Sect. 6.1 and Fig. 1) can measure both neutralizing and non-neutralizing ADAb, depending on whether whole Abs or Fab or F(ab′)$_2$ fragments are used as capture and detection molecules [90]. Interpretation of results from different ADAb assays is complex, and distinction between neutralizing and non-neutralizing ADAb may be difficult unless assay characteristics are described in publications.

5.4 Liquid-Phase Methods

Liquid-phase methods include a high-performance liquid chromatography-based homogenous mobility shift assay [91]. The method is based on size exclusion high-performance liquid chromatography separating the free fluorescent-labeled drug from the ADAb-bound labeled drug. As antigen-antibody binding takes place in the liquid phase, these methods are suggested to circumvent potential interferences related to solid-phase assays [92].

5.5 Functional Assays

Neutralizing ADAb can be measured by reporter gene assays that employ cells carrying nuclear factor-κB regulated firefly luciferase reporter gene constructs activated by TNFα [93]. Presence of TNFi inhibits expression of the reporter gene, and the amount of TNFi is thus inversely proportional to the luminescence produced by the reporter cells. For the detection of neutralizing ADAb, the sample is spiked with a known amount of TNFi, before the inhibition of TNFi

activity is quantified by the luminescence produced by the reporter cells [92]. Functional cell-based assays can be used in combination with immunoassay formats to quantify the amount of both binding and neutralizing ADAb [92]. However, the added value of cell-based assays is unclear, owing to lower sensitivity [94] and because some immunoassay formats are able to selectively detect neutralizing ADAb [95, 96]. The latter is particularly relevant for inhibition assays, but also apply to other immunoassay formats using antibody Fab′ or F(ab′)$_2$ fragments (instead of intact IgG) as solid-phase and/or tracer molecule.

5.6 Drug Interference and Drug-Tolerant Assays

As ADAb detection usually relies on binding to the labeled drug, most assays are d*rug sensitive,* i.e., they have limited ability to detect ADAb in the presence of a circulating drug, owing to the formation of ADAb-drug complexes. *Drug-tolerant* versions have been developed for most traditional ADAb assay formats [85, 97]. In most drug-tolerant assays, serum samples are preincubated in acidic buffer (typically pH < 2.5) to dissociate ADAb-drug complexes before the detection of free ADAb [98, 99]. As these assays can reduce underestimation of ADAb in the presence of a drug and detect ADAb earlier, they can provide insight into the process of ADAb formation [100]. However, their clinical relevance is unclear as the clinical consequences of ADAb primarily depend on whether they neutralize/reduce the amount of pharmacologically active drug to subtherapeutic concentrations. Thus, in the presence of therapeutic concentrations of the free drug, detection of ADAb is rarely clinically relevant [24, 101, 102], as supported by the recent EULAR PtC [10]. In addition, the low pH during the preincubation step may damage some ADAb, which is a concern should such assays be used in clinical practice.

5.7 Rheumatoid Factor Interference

Antibody interference in immunoassays is an important concern as it may confound research and lead to unnecessary and potentially harmful diagnostic and therapeutic interventions in patients [103, 104]. As for other frequently used immunoassays [105], ADAb assays vary in their susceptibility to interference from rheumatoid factor [106]. Rheumatoid factor interference is particularly important to consider when designing immunoassays for the detection of biologic drug concentrations and ADAb, as these assays are intended for use in a high-risk RA population and often rely on binding to human(-ized) immunoglobulins (i.e., biologic drugs). Rheumatoid factor has the potential to cross-link assay antibodies by unspecific binding to the Fc portion in the absence of ADAb. The antibody capture and bridging formats are particularly vulnerable to interference from rheumatoid

factor if not properly protected. The use of antibody fragments (i.e., Fab′ or F(ab′)$_2$ fragments) without Fc, instead of intact IgG, reduces the risk of rheumatoid factor interference and also favors detection of neutralizing ADAb. Other measures to avoid rheumatoid factor interference include the use of blocking reagents and sample dilutions [103]. It is also important to avoid reagent impurities and aggregation [107].

5.8 Assay Comparability

As mentioned earlier, results from different ADAb assays are rarely comparable [94, 108] because of different methodologies, ADAb thresholds, and calibration. In addition to differences in drug tolerance and abilities to detect ADAb with neutralizing potential, assays vary in their ability to detect some isotypes and subclasses of ADAb, and low-affinity ADAb [6, 86, 87, 90]. As immune responses may vary with regard to antibody isotypes, subclasses, and binding properties, ADAb results are often reported in arbitrary units or semiquantitative categories. Calibration against absolute antibody concentrations and reporting of results in mass units (e.g., µg/L) can facilitate an inter-assay comparison, but many assay developers are understandably hesitant given the heterogeneity of ADAb. Given the considerable inter-assay variability, the recent EULAR PtC recommend using the same assay when serial measurements are performed in the same patient for clinical purposes [10]. Antidrug antibodies should also be measured and interpreted alongside contemporaneous drug concentration assessments [10]. The latter is important in order to consider a possible underestimation of ADAb in the presence of the circulating drug, and to evaluate the influence of ADAb on biologic drug pharmacokinetics. Furthermore, we recommend that clinicians familiarize themselves with the assay used and maintain a close dialogue with their laboratory regarding the interpretation of results.

6 Clinical Consequences of ADAb

6.1 Influence on Treatment Outcomes

Neutralizing ADAb decrease biologic activity of the drug by interfering with epitope binding, and both neutralizing and non-neutralizing ADAb form immune complexes that could accelerate drug clearance [6]. As a consequence, ADAb are associated with lower serum drug concentrations and patients may lose response to therapy (Fig. 2) [4, 6, 20, 109]. A relationship between the presence of ADAb assessed by drug-sensitive assays, low serum biologic drug concentrations, and poorer treatment outcomes has been described in prospective observational studies and post-hoc analyses of RCTs for TNFi (adalimumab, infliximab, golimumab, and

Fig. 2 Model of the relationship between anti-drug antibody (ADAb) development, decrease in tumor necrosis factor alpha inhibitor (TNFi) serum concentration, and subsequent loss of clinical response. **a** Free circulating TNFi in sample. **b** Incipient ADAb formation. TNFi and ADAb in complexes. **c** Free ADAb becomes detectable using drug-sensitive assays (when TNFi undetectable/very low). Anti-drug antibodies can presumably be detected earlier using drug-tolerant assays. The clinical effect of the drug diminishes when the concentration of TNFi becomes subtherapeutic (the relative amount of ADAb is higher than free TNFi). Created with BioRender
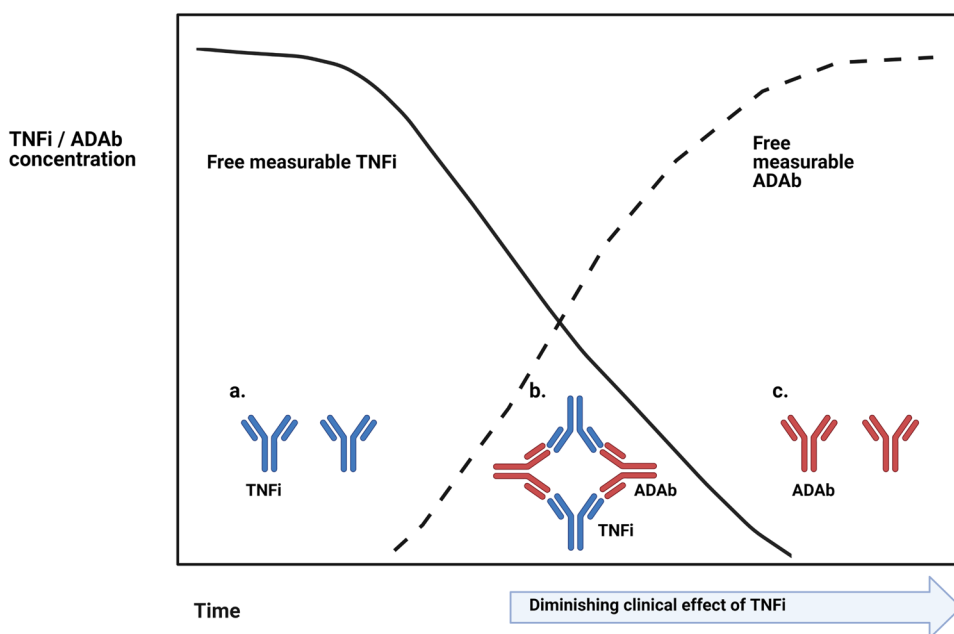
certolizumab) [20, 33, 42, 43, 46, 47, 65, 74, 89, 110–118]. For etanercept, however, an association between ADAb and treatment response likely does not exist [33, 119]. Most studies are performed in patients with RA or SpA. Data regarding other biologic drugs including rituximab, tocilizumab, abatacept, secukinumab, ustekinumab, and ixekizumab are limited or lacking.

However, ADAb are not always associated with a loss of effectiveness and treatment discontinuation, i.e., ADAb assessed by drug-tolerant assays may not influence the drug concentration enough to affect the therapeutic response. Importantly, some ADAb-positive patients may have sufficient serum drug concentrations during the majority of the treatment interval. Additionally, some patients are in remission independent of the drug and are thus unlikely to have a disease flare in the presence of ADAb. Hence, ADAb results should always be interpreted in the context of drug concentrations and the clinical situation [10].

6.2 Influence on Drug Safety

Another important clinical consequence of ADAb development is the increased risk of infusion reactions seen in patients treated with intravenous biologic drugs. Several studies have demonstrated that ADAb to infliximab predispose to infusion reactions, and a meta-analysis has suggested a four-fold increase in infusion reactions to infliximab in patients with ADAb [4]. Data are less robust for other intravenous biologic drugs such as tocilizumab and rituximab [4, 59, 65, 109, 111, 120]. Infusion reactions can range from mild hypersensitivity reactions, including arthralgia and malaise, to more severe infusion reactions, serum sickness,

and rare anaphylactic reactions. Such reactions are likely caused by complexes formed between ADAb and the biologic drug. The role of ADAb in low concentrations, sometimes only detectable by drug-tolerant assays, has not been established, but it has been suggested that high ADAb levels are mandatory for the formation of large complexes necessary for complement activation [28]. There is no evidence linking ADAb to injection-site reactions of subcutaneous biologic drugs [11, 119, 121].

Development of ADAb has also been linked to serious adverse events such as thrombotic events, autoimmune reactions such as lupus-like reactions, and paradoxical events such as drug-induced vasculitis, the evidence for which has been previously reviewed [122]. An early study [123] reported a higher incidence of serious arterial and venous thromboembolic events in adalimumab-treated patients with ADAb (4/76), compared with patients without ADAb (4/196). Whilst highlighting a potential signal for further explorations, the conclusions were limited because there were only eight thrombotic events in total and a number of methodological challenges [122]. Reports from clinical practice suggest associations between ADAb and biologic drugs, adalimumab in particular, and adverse events such as rash, arthralgia, and fever, but no data exist to support this association.

Immune manifestations associated with TNFi medications can vary from a spectrum of asymptomatic immunological variations to clinical autoimmune manifestations. Anti-nuclear antibody formation is commonly seen in patients initiating a TNFi [124, 125]. It has also been proposed that patients receiving TNFi therapy may develop ANA and anti-double-stranded DNA antibodies because of

immunogenicity [84, 126, 127]. Seroconversion of ANA and double-stranded DNA has also been observed at higher rates in patients with RA [125, 128] and psoriasis [126] in association with secondary non-response to TNFi, with a direct association with ADAb seen in infliximab-treated patients [127]. Similarly, in patients with inflammatory bowel disease, perinuclear anti-neutrophil cytoplasmic antibodies positivity has been associated with a lower clinical response in monoclonal antibody-treated patients and with factors associated with the risk of ADAb formation such as monotherapy [129, 130]. Patients predisposed to ADAb formation may also therefore be prone to developing autoantibodies such as ANA, double-stranded DNA, and ANCA. However, in large biologic observational studies in RA, the development of lupus and vasculitis like-events is infrequent with an absolute risk of 1/1000 patient-years (95% confidence interval 8–13) and 15/10,000 patient-years (95% confidence interval 12–19), respectively [130]. More knowledge regarding the association between ADAb formation and adverse events is warranted [10].

7 Role of ADAb Measurement in Personalized Treatment Strategies: TDM

Therapeutic drug monitoring of biologic drugs refers to measurements of serum concentrations of drugs and ADAb to guide clinical decision making. *Proactive TDM* refers to scheduled measurements with subsequent dose optimization, irrespective of the clinical situation, whereas *reactive TDM* refers to measurements in response to particular clinical situations, such as a suspected treatment failure [10]. Suggested algorithms for the interpretation of TNFi serum concentrations and ADAb in proactive and reactive TDM are shown in Fig. 3. Data supporting the clinical value of TDM of biologic drugs are still limited and only derived from TNFi [11].

7.1 Proactive TDM

An individualized treatment strategy based on regular assessments of serum drug concentrations and ADAb, *proactive* TDM, has been proposed as a clinical tool to optimize efficacy, patient safety, and the cost effectiveness of biologic drugs. However, most data are available for TNFi in gastroenterology [88, 131, 132]. Proactive TDM may optimize biologic drug therapy by: (a) minimizing undertreatment, which might lead to a lack of response, a loss of response, and possibly also predispose to ADAb production; (b) reducing overtreatment, which predisposes patients to side effects and increases costs; and (c) allowing for early identification of ADAb development, with the possibility to detect treatment failures prior to a clinical flare, discontinue ineffective treatment, and prevent hypersensitivity reactions.

The NOR-DRUM trials were the first randomized clinical trials to compare the effectiveness of proactive TDM to standard infliximab therapy across patients with immune-mediated inflammatory diseases, including IJDs [88, 133]. The NOR-DRUM A trial including 411 adults with RA, SpA, PsA, ulcerative colitis, Crohn's disease, or psoriasis initiating infliximab therapy showed that proactive TDM compared to standard therapy did not improve treatment outcomes during induction of infliximab therapy, with a comparable proportion of patients (51 vs 53%) achieving remission at week 30 in the TDM and standard therapy group [133]. The study, however, suggested a role of proactive TDM during induction in patients at a high risk of ADAb formation as the proportion of patients in remission was higher in the TDM group (56%) than the standard therapy group (35%), among the 70 (15%) patients who developed ADAb. Furthermore, the results suggested that regular ADAb measurements may prevent infusion reactions, as significantly fewer infusion reactions was seen in the TDM group ($n = 5$) than in the standard therapy group ($n = 16$). The NOR-DRUM B trial, addressing the effectiveness of proactive TDM in the maintenance phase of infliximab therapy included 458 patients with RA, SpA, PsA, ulcerative colitis, Crohn's disease, or psoriasis receiving infliximab therapy, concluded that proactive TDM was more effective than treatment without TDM in maintaining disease control [88]. The primary endpoint of sustained disease control without disease worsening was observed in 167 (73.6%) patients in the TDM group, and 127 (55.9%) patients in the standard therapy group. The adjusted difference was 17.6% (95% confidence interval 9.0–26.2%; $p < 0.001$) favoring TDM. Adverse events were reported in 137 patients (60%) and 142 patients (63%) in the TDM and standard therapy groups, respectively. Twenty-one patients (9.2%) in the TDM group and 27 patients (15.0%) in the standard therapy group developed clinically significant levels of ADAb. In the TDM group, 17 (8%) patients discontinued infliximab because of ADAb. Importantly, drug consumption was similar in both groups. The different results of the two NOR-DRUM trials may be due to different mechanisms for lack of response/primary treatment failure during the induction period and loss of response/secondary treatment failure during maintenance therapy [134]. Low drug concentrations may induce therapeutic failure during the maintenance phase, whereas high drug exposure may diminish potential benefits of TDM during the induction phase [135].

The NOR-DRUM trials suggest that proactive TDM may be beneficial during maintenance infliximab therapy, but not during induction unless the patient is at a high risk of developing ADAb. Risk factors of ADAb formation are described in Sect. 5. The potential benefit of proactive TDM should
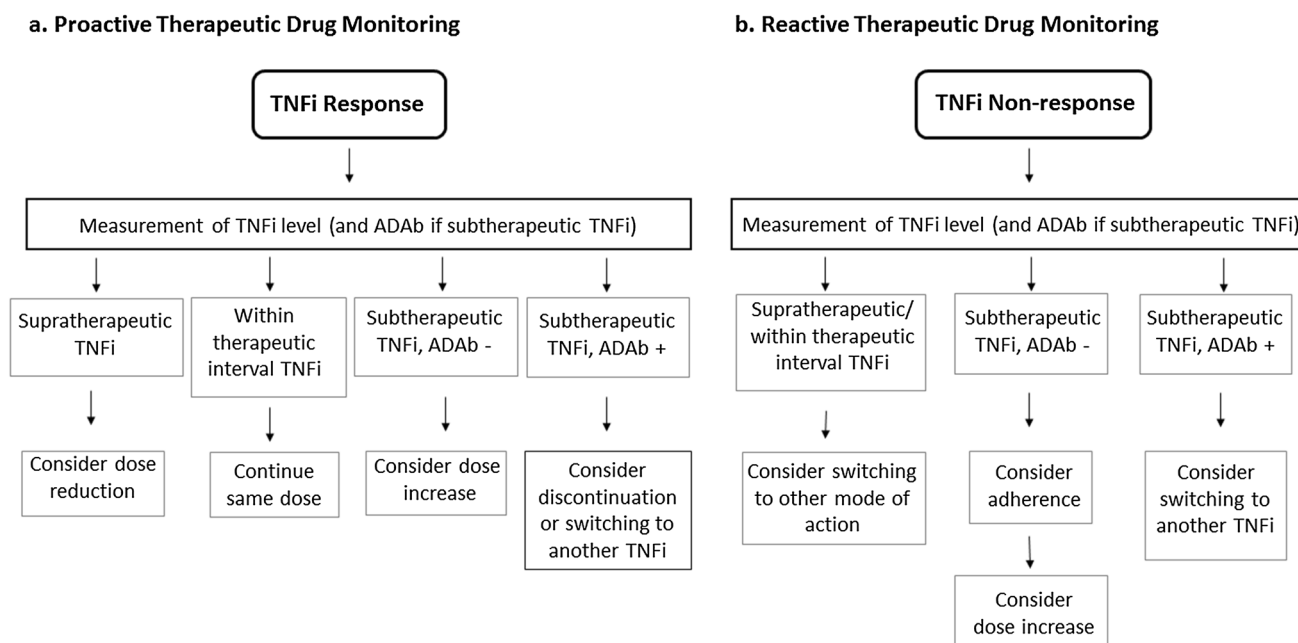
a. Proactive Therapeutic Drug Monitoring

b. Reactive Therapeutic Drug Monitoring

Fig. 3 Suggested algorithm for the interpretation of tumor necrosis factor inhibitor (TNFi) levels and anti-drug antibodies (ADAb) in responders or non-responders to TNFi. **a** Proactive therapeutic drug monitoring; regular measurements with subsequent dose optimiza-

tion and **b** reactive therapeutic drug monitoring; measurements in response to particular clinical situations, such as a suspected treatment failure

be balanced against the costs of performing TDM [136], which may be both direct and indirect costs. Avoiding disease worsening has important implications for patients [137, 138], as well as inflicting a burden on healthcare systems and financial implications for society. Cost-effectiveness analyses are needed to further explore this issue. Because of the immunogenicity profile of infliximab, the results of the NOR-DRUM trials cannot be extrapolated to other TNFi or other biologics, and additional randomized clinical trials are needed to clarify a benefit of TDM for these biologics. Based on limited evidence, the EULAR PtC developed prior to the NOR-DRUM B study did not recommend routine proactive TDM in the management of IJDs [10]. Prospective studies comparing TDM with standard of care with regard to clinical utility and cost effectiveness of TDM, for a range of biologic drugs in different IJDs, are still needed.

7.2 Reactive TDM Strategies

7.2.1 Treatment Failure

Reactive TDM is suggested as a tool to understand the cause of treatment failure, and can guide treatment decisions [10, 139], as shown in Fig. 3. In choosing between increasing the dose of the current biologic drug or switching to another therapy in the case of low drug concentrations, a dose increase may be the most appropriate choice in the absence of ADAb (Fig. 3) [88, 140]. As ADAb may be transient [24],

some clinicians advocate a dose increase also in the presence of ADAb. Low to moderate levels of ADAb can sometimes be overcome, at least temporarily, by increasing the dose of the biologic drug. However, this treatment strategy has not been supported by observational data in infliximab-treated patients with RA and SpA with ADAb [111, 141]. These studies showed that although ADAb levels decreased temporarily following dose escalation in some patients, in most patients ADAb levels continued to increase despite the intervention [111, 141]. A reduction in ADAb levels in response to dose increase was primarily observed in patients with low ADAb levels [141]. These data suggest that ADAb perceived as transient are truly transient only in a minority of cases, whereas in most cases ADAb are "hidden" by higher drug concentrations and remain undetected in drug-sensitive assays [24]. It remains unclear whether adding or increasing the dose of concomitant synthetic disease-modifying antirheumatic drugs can mitigate an ongoing ADAb response.

Antidrug antibodies against a TNFi do not cross-react with other TNFi (except the corresponding biosimilars). It is thus appropriate to switch within the class in the context of ADAb to first TNFi (Fig. 3). Observational data have suggested that ADAb formation to one TNFi predicted response to the subsequent TNFi in patients with RA and SpA [142–144], but results have been conflicting [145, 146]. It has, however, been suggested that patients who develop ADAb to first TNFi have an increased risk of developing de novo ADAb to the next TNFi [47, 147]. This probably

reflects common risk factors/mechanisms behind ADAb development to different TNFi as described above. The effect of switching to less immunogenic etanercept in the case of ADAb remains unclear [10, 142, 145].

Reactive TDM has been applied in clinical practice by many rheumatologists, based on empirical and observational data. The recent EULAR PtC state that reactive TDM, including both ADAb and drug concentrations, should be considered to understand clinical non-response, but this statement is based on limited data [10, 11]. Clinical trials addressing the effectiveness of reactive TDM strategies in the case of a suspected treatment failure are warranted.

7.3 Other Clinical Situations

7.3.1 A Biomarker to Predict Response

Observational studies have indicated that early assessment of ADAb status (weeks 4–14), in combination with drug concentrations, can predict later non-response to TNFi [10, 23, 148–150]. However, the clinical value of ADAb as an early biomarker to predict non-response, must be further investigated.

7.3.2 Tapering

Tapering of biologic drugs is a topic of interest, and assessment of drug concentrations and ADAb may help identifying candidates for tapering and/or discontinuation. Patients in stable remission despite subtherapeutic TNFi concentrations (with or without ADAb) are likely to be in spontaneous remission, and may probably taper or discontinue treatment without risking a disease worsening. However, current data for the utility of such clinical strategies are conflicting, and further studies are needed [116, 151–153]. Also relevant in the context of tapering, low drug exposure has been linked to an increased risk of ADAb development [5, 45, 65], thus monitoring patients with drug concentrations and ADAb might be beneficial during tapering. Monitoring of ADAb is also relevant when restarting the same biologic drug, as drug holidays are associated with an increased risk of ADAb development [5, 45].

7.3.3 Infusion/Hypersensitivity Reactions

Because of the known relationship between ADAb and infusion/hypersensitivity reactions to infliximab [4], measurement of ADAb is often performed if a hypersensitivity reaction is suspected, in line with the EULAR PtC [10]. One could argue that in the case of a severe reaction, it seems appropriate to discontinue infliximab regardless of ADAb status. However, in the case of a milder or unspecific reaction, measuring ADAb might aid in further treatment decisions.

7.3.4 Non-medical Switching to Biosimilars

Despite reassuring evidence regarding the effectiveness and safety of switching among biosimilars and the reference product, including the NOR-SWITCH trial [154, 155], there are still concerns among some clinicians and patients that switching to a new biosimilar may result in disease flares [156]. Monitoring ADAb status may be helpful in this situation, for instance to assess whether an adverse event can be attributed to immunogenicity of the biosimilar. If ADAb to the reference product is present, switching to a corresponding biosimilar is not recommended because of the cross-reactivity of ADAb to biosimilar products [157, 158]. Based on current evidence, biosimilars can be expected to exhibit the same immunogenicity as the originator molecule [159]. Comparative immunogenicity studies are required by the European Medicines Agency and the US Food and Drug Administration as part of the approval process of biosimilars and data on immunogenicity are presented in the relevant European Public Assessment Reports [158].

8 Future Potential and Challenges of ADAb Assessments in Personalized Treatment

Biologic drugs are at the core of modern treatment strategies for rheumatic diseases, and their global use is increasing. Assessing serum drug concentrations and ADAb formation against these agents offers an opportunity to personalize treatment, which has long been a goal within modern medicine. To achieve this, however, several barriers must be overcome. High-quality clinical trials of TDM within rheumatology are lacking, except for the NOR-DRUM trials [11]. This knowledge gap is underlined by the EULAR PtC research agenda [10]. The conduct of such trials requires that the therapeutic range of the biologic drug investigated has been identified. With the exception of infliximab and adalimumab, therapeutic ranges remain largely undetermined [11]. Reliable markers identifying patients at high risk of ADAb formation will help clinicians make treatment decisions to minimize such risk. Though progress has been made, including research on HLA markers, reliable predictors for use in clinical practice are still lacking. Cost-effectiveness studies on TDM are much needed, but also difficult to carry out, among other reasons because the cost of biologic drugs and testing vary from region to region and over time [10, 11]. Anti-drug antibody assay heterogeneity will remain a challenge for the foreseeable future. Initiatives such as Abirisk [160] have sought to form regional consortia to harmonize ADAb assays. Further efforts to help clinicians navigate the

landscape of widely different ADAb assays should be welcomed. Home-sampling techniques and point-of care rapid tests have been developed for drug concentration and ADAb testing and could facilitate implementation of TDM in clinical practice [161–165]. Further research into immunogenicity, potential benefits of ADAb monitoring, and clinically validated ADAb assays are required to support TDM as a management approach in the future. Finally, there remains a need to educate physicians in the use of serum drug concentrations and ADAb measurements and identify barriers to the implementation of TDM [10, 166].

9 Conclusions

Assessing ADAb in combination with drug concentrations has shown promise as a strategy to optimize treatment with biologic drugs widely used in the treatment of IJDs today. Several challenges must be overcome for these assessments to be widely implemented in clinical rheumatology. In particular, identifying therapeutic drug concentration ranges and guidance regarding the interpretation of drug concentrations and ADAb results in the context of the clinical situation are essential. Importantly, high-quality clinical trials assessing clinical utility and cost effectiveness of assessments of ADAb and drug concentrations, both reactive and proactive TDM, are needed to form the basis for future guidelines and recommendations in rheumatology.

Declarations

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Author contributions JEG: Review design; drafting and revision manuscript; preparation of figures. GLG: review design; drafting and revision of manuscript. MKB: Drafting and revision of manuscript. MJ: Drafting and revision of manuscript. NB: Review design; drafting and revision of manuscript. SWS: Review design; drafting and revision of manuscript.

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