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Reporting of quality attributes in scientific publications presenting biosimilarity assessments of (intended) biosimilars: a systematic literature review

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ABSTRACT

Last years, more than 46 unique biosimilars were approved by EMA and/or US-FDA following patent expiration of reference products. Biosimilars are not identical like generics, but highly similar versions where demonstrating biosimilarity of quality attributes (QAs) to a reference product is the basis of development and regulatory approval. Information on QAs assessed to establish biosimilarity may not always be publicly available, although this information is imperative to understand better the science behind biosimilars approval. This study aims to identify QA types reported in publications presenting biosimilarity assessments of (intended) biosimilars over time. English full-text publications presenting biosimilarity assessments of QAs for (intended) biosimilars between 2000 and 2019 identified from PubMed and EMBASE. Publication characteristics and QAs classified into: structural (physicochemical properties, primary structure, higher-order structures (HOSs), post-translational modifications (PTMs), and purity and impurities) and functional (biological and immunochemical activities) were extracted from publications. Seventy-nine publications were identified (79% open-access, 75% industry-sponsored, 62% including unapproved biosimilars, and 66% involving antibodies). Reporting frequencies varied for QA types: biological activity (94%), physicochemical properties (81%), PTMs (79%), primary structure (77%) purity and impurities (73%), HOSs (58%), and immunochemical activity (41%). The number of publications increased from 6 (7%) during 2009–2011 to 62 (79%) during 2015–2019. Eighteen (28%) publications reported all QA types relevant to an active-biological-substance. Reporting of most QA types increased over time that most evidenced by immunochemical activity (from 0% to 47%) which occured after EMA monoclonal antibody (mAbs) guidline in 2012 and more publications on mAbs later on when compared to earlier period. Biosimilarity assessments of QAs have been published in peer-reviewed publications for about 60% of approved biosimilars. Publishing biosimilarity assessments and reporting QAs over time appears to be affected by regulatory actions that occurred in 2012-2015, including regulatory approval and development of regulatory guidelines for biosimilars. Availability of a complete, publicly accessible and unbiased biosimilarity assessment of QAs, as part of a trusted and transparent regulatory process, will contribute to increased confidence and acceptance of biosimilars in clinical practice.

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1. Introduction

Recombinant DNA technology has enabled the production of therapeutic proteins as effective, mechanism-based treatments for a variety of diseases (Avidor et al., 2003). Since the first recombinant human insulin was granted regulatory approval in 1980 (Johnson, 1983), multiple generations of recombinant DNA therapeutics ranging from single polypeptide chains such as hormones and cytokines to substantial and complex coagulation factors and antibodies have been developed and have received regulatory approval (Walsh, 2000; 2003; 2006; 2010; 2014; 2018). Biologicals offer important treatment options and accounted for 47% of all medicinal products containing novel molecular (chemical or biological) entities that were approved between 2014 and July 2018 in the US (Walsh, 2018).

As patents of several biologicals have expired, the door opened for the development of subsequent versions: the so-called biosimilars or follow-on biologics. The first regulatory pathway for the approval of biosimilars was developed in 2005 by the European Medicines Agency (EMA) (EMA, 2014; EMA, 2015). Subsequently, after years of debate, the US Food and Drug Administration (US-FDA) launched an abbreviated regulatory pathway for biosimilars in 2015 (US-FDA, 2015a; 2015b). These biosimilar regulations intended to enable wider and earlier patient access for important medicines and to realize remarkable cost savings to reduce pressure on health care budgets (McCamish and Woollett, 2012; Aladul et al., 2017). Up to this date, no product-specific safety and/or efficacy concerns were identified in clinical practice for licensed biosimilars in Europe supporting the robustness of the current regulatory framework (Vermeer et al., 2019; Cohen et al., 2018; McKinnon et al., 2018)

In contrast to chemically synthesized generics that are identical copies, biosimilars are highly similar versions with respect to quality characteristics, biological and clinical activity, safety, and efficacy of the previously authorized reference products. The comparability assessment of quality attributes (QAs) between a biosimilar and the reference product is the basis for establishing biosimilarity during the development and regulatory approval of biosimilars. Quality attributes are measurable product characteristics that describe the physical, chemical, biological, and microbiological properties of a drug molecule (ICH, 2009). In contrast to chemical drugs, biologicals are large and, often, complex molecules produced by living systems. This, and the complexity of the molecular structure and production process for biologicals results in a drug molecule with intrinsic variability (isoforms) and subsequent variability in QAs. The QAs of biologicals are heterogeneous and susceptible to changes in production processes that may, intentionally or not, for the same product result in gradual or sudden changes over time (Vezér et al., 2016; Vulto and Jaquez, 2017). Thus, variability in QAs of all biologicals is inevitable between batches derived from the same process; even isoforms in a single batch hardly remain constant over (storage) time (Schiestl et al., 2011; Planinc et al., 2017; Kim et al., 2017).

Demonstrating biosimilarity requires a stepwise comparability assessment between a biosimilar and a reference product. The comparability of QAs is the mainstay for detecting potential differences and establishing biosimilarity. As a result of the advancement in science and analytical technology, the comparative efficacy (phase III) trials became less important for certain product classes such as filgrastim, teriparatide and insulin biosimilars (EMA, 2015; Wolff-Holz et al., 2019).

Over the last decade, more than forty-six unique biosimilars (> eighty-seven brand names) have received regulatory approval from the EMA and/or US-FDA and this number is expected to further increase over the coming years. Although there is a robust and reliable regulatory framework for the approval of biosimilars, the uptake of and trust in biosimilars in the US and some European countries is still very low (European Commission, 2019; GaBI, 2019; Rathore et al., 2019). Clinicians focus on clinical trial data whereas the regulatory approval of biosimilars heavily relies on the comparability/biosimilarity assessment of QAs. The selection of QAs needed to establish the biosimilarity is not standardized yet and information on QAs accessible in the public domain is variable and for quite a few products limited. Compendial European and US pharmacopeia monographs cannot be considered as reference because these may not capture all QAs of the reference product, and have not yet been developed for clinical use (Thorpe and Wadhwa, 2011). The information on QAs of biosimilars are documented by the developers in confidential registration dossiers that are not publicly available, and reflected by the regulators in public assessment reports that are generally not well-known by stakeholders (Hallersten et al., 2016). Sharing information on QAs through peer-reviewed scientific publications in a transparent manner is imperative to better understand the science behind the regulatory approval of biosimilars. As the clinical profile of biological drugs is influenced by their structural and functional QAs, information on QAs also results in better understanding on the role of QAs on clinical parameters. Previous systematic reviews show that there is a substantial discordance in the extent of published evidence to support establishing the biosimilarity between biological molecules across therapeutic areas[31-33]. However, no overview on the types of QAs studied in scientific publications to establish the biosimilarity is available in literature. Therefore, this study aims to identify the types of QA reported in scientific publications and provide an overview of the dynamics of scientific publications presenting biosimilarity assessments of QAs for (intended) biosimilars over time.

2. Methods

2.1. Systematic literature search

PubMed and EMBASE databases were used to collect scientific publications in peer-reviewed journals that presented biosimilarity assessments (i.e. analytical comparison) of QAs for (intended) biosimilars. The word “intended”, hereafter, refers to any version of a recombinant therapeutic protein that was not approved by the EMA or US-FDA as a biosimilar at the date of publication. A search strategy was constructed to systematically identify relevant scientific publications between January 1, 2000 and December 31, 2019. Search strings were created to include indexed terms and controlled keywords that were selected to define a search domain, determinant, and outcomes. The search formula (Domains AND (Determinants OR Outcomes)) was applied, which is provided in **Supplementary Table-S1a-b**. Screening of titles and abstracts was performed to verify the search strings. The search filter title/abstract was used to retrieve publications pertinent to the study objective. The search strings were executed on May 21, 2018 and were refreshed on January 1, 2020 to capture recently indexed scientific publications up to December 31, 2019. This search was conducted according to PRISMA guidelines (Moher et al., 2009).

2.2. Inclusion criteria

Duplicate publications identified by the search strategy were removed by the first author (AMA). The titles and abstracts of identified publications were screened by the same author to identify relevant and eligible full-text publications, which were further categorized into primary source “original publication” and secondary source “review publication”. The list of references of each review publication was manually checked by AMA to retrieve relevant publications that were not captured in the electronic searches. If there was doubt about the eligibility of a publication for inclusion, a consensus decision was reached after discussion between AMA, TJG, and HG. Publications were considered eligible when: (I) the full-text article was in English; (II) (intended) biosimilars were assessed; (III) the active biological substance of the reference (comparator) was clearly defined; and (IV) a comparability/biosimilarity assessment (i.e. analytical comparison) of QAs between and (intended) biosimilar and the reference product was

presented. Review publications were excluded unless original data were presented. Publications that assessed (intended) biosimilars containing non-recombinant proteins such as human albumin or heparin were excluded. Publications presenting comparability assessments with the primary aim to show assay suitability or manufacturing capability were excluded. Conference abstracts, preclinical animal studies, and all types of clinical trials were also excluded. European public assessment reports (EPARs) and chemistry review reports of approved biosimilars published by the EMA or US-FDA, respectively, were not considered in this study.

2.3. Characteristics of included scientific publications

Baseline characteristics of each included publication were registered, namely the date of publication, the access-status of the publication, the source of funding, the regulatory-status of the (intended) biosimilar at the date of publication, and the type of active biological substance. The date of publication was the calendar month and year at the time of (first online) publication, which was divided into three periods. The three periods were selected based on the year of first publication and time frames where relevant regulatory guidelines were published and updated by the EMA (2012) and US-FDA (2014/2015), and defined as 2009–2011, 2012–2014, and 2015–2019. The publication access-status was defined as open or non-open access publications. The source of funding was categorized into an industry or academic sponsorship. If the source of funding was not clearly stated, the institution of the corresponding author was considered as the source of funding. The regulatory-status of the (intended) biosimilar was defined as either approved or unapproved on the basis of the regulatory approval from at least one of the stringent regulatory authorities (SRAs) at the date of publication, which were identified from the official websites of the EMA [https://www.ema.europa.eu/en] and the US-FDA [https://www.fda.gov/]. The active biological substance of the (intended) biosimilars was classified into three types: antibodies, hormones, and others such as clotting factors and enzymes.

2.4. Quality attributes

A classification scheme for the QA types was developed based on information about QAs included in biosimilarity assessments as outlined in the EMA and US-FDA biosimilar guidelines (EMA, 2014; US-FDA, 2015a). The constructed classification scheme was discussed with regulators involved in the quality assessment of biosimilars at the Dutch Medicines Evaluation Board (MEB) [https://english.cbg-meb.nl/]. The QAs were first classified into structural or functional QAs, which included seven types of QAs in total. The structural QAs included five types: physicochemical properties, primary structure, higher-order structures (HOSs), post-translational modifications (PTMs), and purity and impurities. The PTMs were further divided into two subtypes: enzymatic PTMs including glycosylation and non-enzymatic PTMs. The purity and impurities were divided into two subtypes: size and charge variants. The functional QAs included two types: biological and immunochemical activities. All QA types included in the classification scheme are relevant to recombinant therapeutic protein with the exception of the enzymatic PTMs and the immunochemical activity that are only specific to glycoproteins and monoclonal antibodies and fusion proteins, respectively **(Box 1)**.

2.5. Data analysis

All reported QAs in the scientific publications were extracted, analyzed, and assessed using descriptive statistics. From each publication, the reported QAs were identified and sorted according to the developed classification scheme for the QA types **(Box 1)**. The reporting frequencies were calculated for each QA type and subtype, which were stratified by the characteristics of the publication: publication date, funding source, regulatory-status, and active biological substance(s) type of the (intended) biosimilar(s). The median number for the reported QA types in publication(s) per year was calculated to present the dynamics of reporting QA types over time. For biosimilars that were approved by EMA or US-FDA, the pertinent scientific publications were identified for each unique biosimilar per the company development code or, if not applicable, per brand names. If a biosimilar granted approval from both agencies, the first regulatory approval date was considered to calculate the time difference (in calendar months) between the date of publication and regulatory approval. Follow-up ended on December 31, 2019. The statistical calculations were conducted using IBM SPSS 25 Statistical software (SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1. Systematic literature search and characteristics of included publications

The search identified 1159 scientific publications that were potentially eligible for inclusion. After removing the duplicates, a total of 1012 publications were identified, of which 933 were excluded after title/abstract screening, most of which were conference abstracts or publications not related to biosimilarity assessments of QAs for (intended) biosimilars. This resulted in 79 full-text publications eligible for inclusion and further analysis **(Fig. 1)**.

The baseline characteristics of the 79 included publications (Park et al., 2009; Skrlin et al., 2010; Sorgel et al., 2010; Brinks et al., 2011; Maity et al., 2011; Meager et al., 2011; Tan et al., 2012; Visser et al., 2013; Crobu et al., 2014; da Silva et al., 2014; Flores-Ortiz et al., 2014; Halim et al., 2014; Hurst et al., 2014; Jung et al., 2014; Rathore and Bhambure, 2014; Ryan et al., 2014; Sadeghi et al., 2014; Escobedo-Moratilla et al., 2015; Lopez-Morales et al., 2015; Miranda-Hernandez et al., 2015; Miranda-Hernandez et al., 2015; Sorgel et al., 2015; Chen et al., 2016; Cho et al., 2016; Cuello et al., 2016; Derzi et al., 2016; Halim et al., 2016; Hausberger et al., 2016; Hofmann et al., 2016; Holzmann et al., 2016; Huang et al., 2016; Liu et al., 2016; Mendoza-Macedo et al., 2016; Miranda-Hernandez et al., 2016; Nupur et al., 2016; Velayudhan et al., 2016; Zhao et al., 2016; Brokx et al., 2017; Hong et al., 2017; Lee et al., 2017; Lim et al., 2017; Magnenat et al., 2017; Mastrangeli et al., 2017; Miao et al., 2017; Montacir et al., 2017; Strand et al., 2017; Velasco-Velazquez et al., 2017; Vorob'ev et al., 2017; Winstel et al., 2017; Hassett et al., 2018; Jeong et al., 2018; Kronthaler et al., 2018; Lee et al., 2018; Lee et al., 2018; Montacir et al., 2018; Montacir et al., 2018; Morimoto et al., 2018; Nupur et al., 2018; Peraza et al., 2018; Seo et al., 2018; Singh et al., 2018; Thennati et al., 2018; Beyer et al., 2019; Lee et al., 2019; Hutterer et al., 2019; Jassem et al., 2019; Korn et al., 2019; Kovacs et al., 2019; Lee et al., 2019; Lee et al., 2019; Paek et al., 2019; Shekhawat et al., 2019; Kang et al., 2019; Kang et al., 2020; Cerutti et al., 2019; An et al., 2019; Xu et al., 2019; Fazel et al., 2019) are described in Table 1. A large proportion of the included publications were open access (79%) and funded by the industry (75%). Thirty of the included publications (38%) studied biosimilars that had received regulatory approval at the date of publication. Most of the included publications presented biosimilarity assessments for (intended) biosimilars containing antibodies (66%).

3.2. Reporting of quality attributes over time

Reporting of QAs varied between publications where the biological activity (94%) and physicochemical properties (81%) were the most frequently reported QA types. When comparing the reporting of QA types between publications, it was found that physicochemical properties (92% unapproved versus 63% approved), and primary structure (86% unapproved versus 63% approved) were often reported in publications of unapproved biosimilars, whereas immunochemical activity

Structural quality attributes	Functional quality attributes

• **Physiochemical properties**
• **Primary structure**
• **Higher order structures-HOSs**
• **Post-translation modifications-PTMs**
  • Enzymatic- PTMs*
  • Non-enzymatic-PTMs
• **Purity and impurities**
  • Size variants
  • Charge variants
	• **Biological activity**
	• **Immunochemical activity***

Box 1. A constructed classification scheme for the quality attribute types in biosimilarity assessment of biosimilars. * Enzymatic-PTMs and Immunochemical activity only applies to glycoproteins and antibodies, respectively.

(50% approved versus 35% unapproved, and 25% academia versus 46% industry) were often reported in publications of approved biosimilars and publications sponsored by industry. The majority of publications sponsored by industry (48 out of 59; 81%) and publications of studied approved biosimilars (18 out of 30; 60%) included biosimiliarity assessment of QAs for antibodies. Sixty-five of the included publications (82%) assessed (intended) biosimilars containing glycoproteins, for which enzymatic PTMs (e.g., glycosylation) are of relevance. In these 65 publications enzymatic PTMs were reported in 78%. The enzymatic PTMs were more often reported in publications of (intended) biosimilars containing antibodies, which accounted for 52 out of 65 (80%) of publications for (intended) biosimilars containing glycoproteins.

Most of the QAs were more frequently reported over time when comparing the periods 2009–2011 and 2015–2019—from 50% to 79% for primary structures, 67% to 82% for PTMs, 50% to 63% for HOSs, and 0% to 47% for immunochemical activity while reporting of some other QAs slightly decreased over time—from 100% to 71% for purity and impurities, 100% to 76% for general physicochemical properties, and 100% to 94% for biological activity—. Interestingly, reporting of immunochemical activity was first noted in the period 2012-2014 where reporting increased from 27% to 47%, which was in parallel with the increase of the number of publications and approvals for (intended) biosimilars of antibodies for which immunochemical activity is relevant (**Table 2**).

Of the included 79 publications, 24 (30%) reported all QA types that are relevant to the active biological substance of (intended) biosimilars being studied. The number of publications that reported all relevant QA types increased from 1 (17%) out of 6 publications during 2009-2011 (median = 4.0 QA types) to 21 (34%) out of 62 publications during

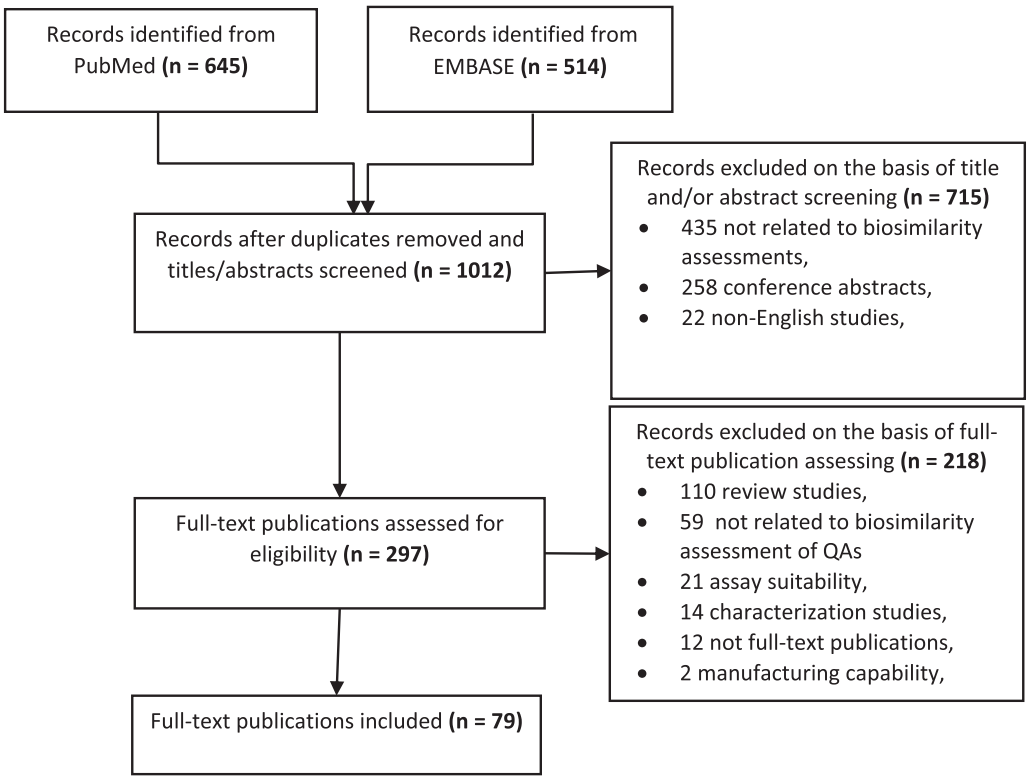

Fig. 1. Flowchart depicting the inclusion criteria of eligible full-text scientific publications.

Table 1
Baseline characteristics of the included scientific publications.

Baseline characteristics	Publications n = 79 (100%)
Publication date	
2009–2011	6 (7%)
2012–2014	11 (14%)
2015–2019	62 (79%)
Access-status of publications	
Open-access	62 (79%)
Non-open access	17 (21%)
Funding source	
Academia/Public	20 (25%)
Industry/Private	59 (75%)
Regulatory status of (intended) biosimilars at the date of publication	
Approved	30 (38%)
Unapproved	49 (62%)
Types of active biological substance of (intended) biosimilars	
Antibodies	52 (66%)
Hormones	24 (30%)
Others (e.g., clotting factor and enzyme)	3 (4%)

2015-2019 (median = 6.0 QA types) **(Fig. 2)**.

3.3. Dynamics of scientific publications of (intended) biosimilars over time

The first scientific publication that presented a biosimilarity assessment of QAs of (intended) biosimilars was published in 2009 while the first open-access publication was found in 2011. The number of scientific publications presenting biosimilarity assessments of QAs increased from 6 (7%) publications in the first period 2009–2011 to 11 (14%) publications in the second period 2012–2014 and 62 (79%) publications in the last period 2015–2019 **(Fig. 3)**.

The first period (2009–2011) included biosimilarity assessments for (intended) biosimilars containing hormones while more complex (intended) biosimilars containing antibodies became available in latter periods. These publications presented biosimilarity assessments of QAs for (intended) biosimilars against reference products for 19 distinctive active biological substance(s). The number of publications for monoclonal antibodies varied and ranged from 1 for *tocilizumab* to 14 for 18 (intended) biosimilars of *rituximab*. Most of the hormones were supported with a single publication, except for 23 *filgrastim, 14 epoetin* and 3 *follitropin alfa (intended) biosimilars, which* were supported with 9, 4, 3 publications, respectively (**Supplementary Figure-S1**).

During the study period, the increase in the number of publications was in parallel with the increase of regulatory approval of biosimilars by the EMA and US-FDA **(Fig. 3)**. As of December 2019, the EMA and US-FDA have approved 46 unique biosimilars with 87 brand names as alternative to 15 reference products (**Supplementary Table-S2**). More than half (56%) of the 46 unique biosimilars have at least one published biosimilarity assessment of QAs presented in a total of 36 publications where the majority (n = 33, 92%) was available as open-access publications. The remaining 43 publications studied (intended) biosimilars that were not yet approved by EMA and/or US-FDA as of December 2019. The overall average duration of time from the date of regulatory approval until publishing the first biosimilarity assessment of QAs for approved biosimilars was twelve months (SD = 27 months). Time from date of regulatory approval until scientific publication of the first biosimilarity assessment was the longest for biosimilars containing hormones (average mean = 33 months, SD = 22 months) after approval and shortest for biosimilars containing antibodies (average mean = 2.5 months, SD = 19 months) before approval **(supplementary Figure-S2).**

4. Discussions

Our study found that reporting frequencies of the QA types in biosimilarity assessments of (intended) biosimilars varied among the included scientific publications. The most frequently reported QA types were the physicochemical properties and biological activity as these provide first and last insights, respectively, into the (dis)similarity between the (intended) biosimilar and the reference product at the molecular level. Reporting of most QA types increased over the study period, specifically the immunochemical activity that was reported after the publication of the EMA guidance entitled "*guideline on similar biological medicinal products containing monoclonal antibodies* (mAbs)–*non-clinical and clinical issues"* in 2012 (EMA, 2012). Although only 26 of the 46 unique biosimilars that have received regulatory approval, as of December 2019, from the EMA and/or US-FDA have a biosimilarity assessment of QAs in a scientific publication, the number of publications has increased over time; furthermore, only one-third of included publications reported all QA types that are relevant to the active biological substance of (intended) biosimilars being assessed.

A large variability in the completeness of reporting the QA types between publications was found, while demonstrating the biosimilarity would require assessing all QA types that are relevant to the active

Table 2
Reporting of quality attribute types in scientific publications on biosimilarity assessments (n = 79), according to date of publication, source of funding, regulatory status of (intended) biosimilar on the date of publication, and type of therapeutic protein for the (intended) biosimilar.

	Total	Publication date			Funding sources		Regulatory status		Types of active biological substance		
	n = 79 (%)	2009-2011 n = 6 (%)	2012-2014 n = 11 (%)	2015-2019 n = 62 (%)	Academia n = 20 (%)	Industry n = 59 (%)	Approved n = 30 (%)	Unapproved n = 49 (%)	Antibodies n = 52 (%)	Hormones n = 24 (%)	Others n = 3 (%)
Structural quality attributes											
Physicochemical properties	81	100	100	76	85	80	63	92	77	88	100
Primary structure	77	50	82	79	80	76	63	86	79	75	67
Higher-order structures-HOSs	58	50	36	63	55	59	57	59	56	63	67
Post translation modifications-PTMs	79	67	64	82	85	76	73	82	79	79	67
Enzymatic PTMs	61	50	46	65	65	59	53	65	75	29	67
Non-Enzymatic PTMs	56	17	55	60	70	51	47	61	54	63	33
Purity and impurities	73	100	73	71	80	71	67	78	67	83	100
Size variants	68	100	73	65	65	70	63	71	62	83	67
Charge variants	57	50	36	61	50	59	57	57	62	46	67
Functional quality attributes											
Biological activity	94	100	91	94	95	93	93	94	94	92	100
Immunochemical activity	41	0	27	47	25	46	50	35	62	NA	NA

*Enzymatic- PTMs and Immunochemical activity only applies to glycoprotein and antibodies, respectively

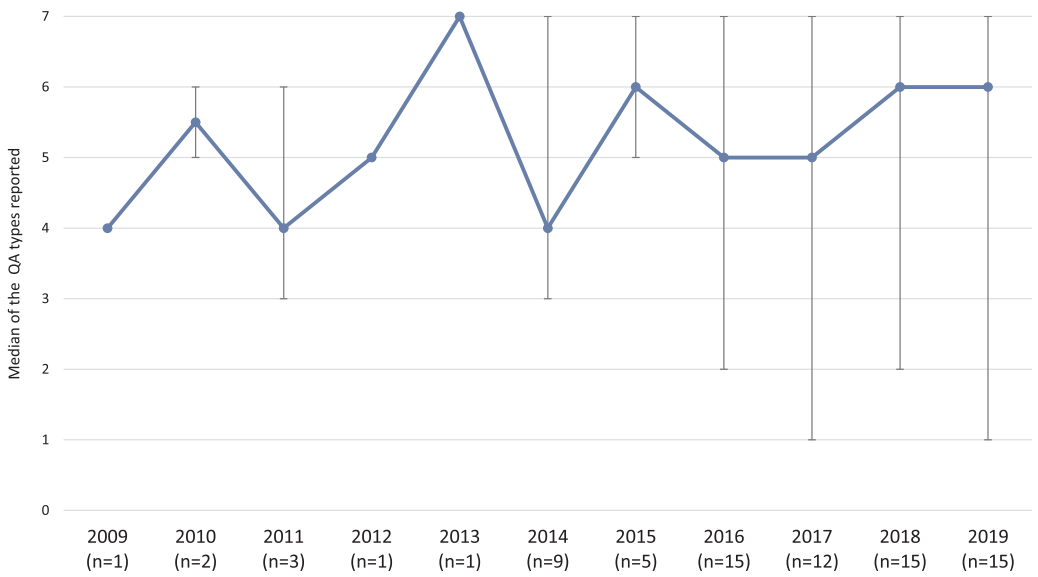

Fig. 2. The median, minimum, and maximum values of the number of quality attribute types reported in the included publications over time.

biological substance of an (intended) biosimilar (EMA, 2014; US-FDA, 2015a). To illustrate this, the enzymatic PTMs (e.g. glycosylation) and immunochemical activity are specific to (intended) biosimilars containing glycoproteins and antibodies, respectively, but were not reported in all pertinent publications. However, the variability in reporting QAs is likely to be driven by the relevance of the QA type for the type of protein. For example, low reporting frequencies of enzymatic-PTMs in publications of hormones is likely due to the fact that hormones, in most cases, are non-glycoproteins where no existence of glycosylation precursors exist. The variability in reporting QA types might also be due to spreading out information on QAs in more than one publication where a few biosimilars have multiple biosimilarity assessments of QAs presented in different publications.

The foundation of establishing biosimilarity is the comparability assessment of QAs between a biosimilars and the reference product, followed by confirmation of biosimilarity by non-clinical studies, pharmacokinetics (PK), pharmacodynamics (PD) and comparative clinical efficacy and safety data where indicated (Wolff-Holz et al., 2019). The importance of the structural and functional relationship of QAs in establishing biosimilarity is continuously being better understood and characterized with the advancement in science and analytical technology. For example, the primary structure is essential in determining HOSs and might influence biological activity (Kirchhoff et al., 2017). Thus, regulators strictly require an identical amino acid sequences as a matter of principle because different sequence is from a regulatory perspective a different active substance. Alterations in "correct" folding of protein drugs may affect the receptor or antigen binding, and likely may hamper the biological and clinical activity and safety (Berkowitz and Houde, 2014). The PTMs and the purity and impurity QAs including size and charge variants often play a role in the biological activity, and such differences can substantially alter the PK/PD and/or immunogenicity via direct or indirect pathways (Walsh and Jefferis, 2006). Although differences in certain structural QAs can influence functional QAs, differences in functional QAs, including biological and immunochemical activity-for antibodies only-might have an impact on clinical parameters such as the serum half-life or the mode of action(s) (Ghetie et al., 1997; Mori et al., 2007; Kwon et al., 2017). The evaluation of functional QAs can help to predict biosimilarity in the clinical performance and adds important knowledge for extrapolation across therapeutic indications (Weise et al., 2014). Our data show that publications of unapproved biosimilars focused more on physiochemical properties and primary structure as these

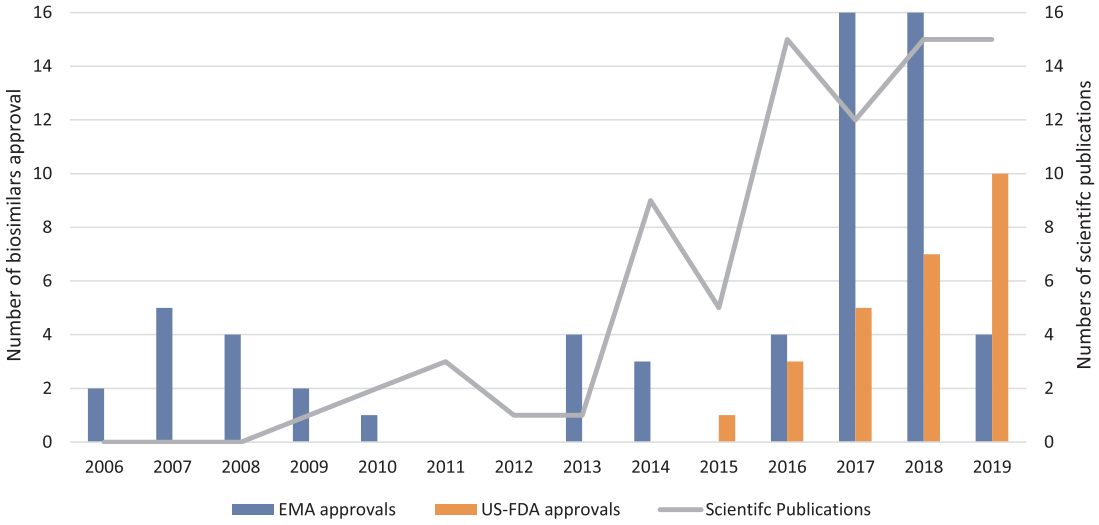

Fig. 3. Dynamics of scientific publications presenting biosimilarity assessments of QAs in relation to the year of regulatory approval of biosimilars by EMA and/or US-FDA.

structural QA types only provide first insights into the biosimilarity between two molecules. On the other hand, the publications of approved biosimilars, which are often mAbs and fusion protein, focused more on biological and immunochemical activity as these types can link with the clinical activity and provide final insights into the bosimilarity at the molecular level. Moreover, the impact of (minor) differences in structural attributes could be assessed by testing functional attributes (Eon-Duval et al., 2012; Kwon et al., 2017; Vandekerckhove et al., 2018) Given the relationships between the QA types and their potential impact on clinical outcomes, it is important to pay equal attention to all relevant structural and functional attributes before concluding the biosimilarity at the quality level. Also, it is essential to report information on all relevant QAs that have or not met predefined biosimilarity criteria. Reporting all QA types relevant to the active biological substance was found in one-third of publications and seemed to increase over time, showing the willingness of publication-sponsors to share a comprehensive biosimilarity assessment of QAs.

The number of publications increased considerably during the study period, although the number is still a marginal fraction of all scientific publication on biosimilars. This positive trend indicates an improvement in knowledge sharing on biosimilarity assessments of QAs, which was not identified in previous systematic reviews (Jacobs et al., 2016; Jacobs et al., 2016; Jacobs et al., 2017). This is perhaps because our search covered a longer time frame and a wider range of protein types, and was specifically designed to identify publications reporting biosimilarity assessment of QAs and assessed the QAs in more details. The increase in publications is likely a direct result of the increased development of biosimilars following patent expiration of reference biologicals by the industry and the growing interest in approval of biosimilars. The patent expiration of reference biologicals played an important role in the timing of arrival to markets where the first wave of approved biosimilars were hormones followed by monoclonal antibodies, the same shifted scope in the protein type of (intended) biosimilar was observed in scientific publications over time. The variabilities in the number of publications between the active biological substances of (intended) biosimilars **(Supplementary Figure-S1)** is consistent with previous findings (Jacobs et al., 2016). The majority of approved biosimilars (75%) were granted regulatory approval between 2015 and 2019, which is in line with the percentage of publications published during the study period (2015-2019). Our data also shows that scientific publications presenting biosimilarity assessments of QAs are available for approximately two-third of the approved biosimilars, revealing a knowledge gap for QAs of some biosimilars in peer reviewed scientific publications. Although the regulatory process has been shown to approve biosimilars which are as safe and efficacious as the reference product, biosimilars still face a sluggish and very low market penetration and uptake in the US and some European countries (European Commission, 2019; GaBI, 2019). Disseminating comprehensive data on biosimilarity assessment, including the QAs, in the public domain is necessary for gaining acceptance of biosimilars among prescribers, payers and patients, thereby achieving sustainable market uptake.

Our data shows some heterogeneity in publishing on QAs between publications that are derived from industry or academic institutions, which is likely explained by the fundamentally different motivations and expectations related to publishing. The motivation of the industry is to develop a biosimilar that meets the regulatory requirements, and as such they always perform a complete assessment of QAs, whereas the academia' immediate unit of success is the publications of what they think is relevant and interesting. The latter might not always include all QAs as what would be expected for an industry driven biosimilar assessment.

The majority of the included publications were funded by industry involved in biosimilar development. In addition, open-accessed publications has increased over time (**Supplementary Figure-S3**), confirming the positive impact of scientific publications about similar drugs on industry rate of publication about its drug in the public domain (Polidoro and Theeke, 2012). This suggests that biosimilar developers are more willing to share the results of their biosimilarity assessment of QAs through open-access publications with the scientific and medical community. Transparency in publishing of comprehensive and unbiased biosimilarity assessment of QA data contributes to better understanding of the science behind regulatory approval and may increases confidence in biosimilars in medical practice. Academic institutions sponsored fewer publications in our review when compared to the industry. This might relate to their limited capacity and resources e.g., facilities and equipment, although three of the academia sponsored publications included in our review reported all relevant QA types to the active biological substance of (intended) biosimilars being assessed. Other factors that play a role in limiting the academic contribution are intellectual property rights and inaccessibility to batches of biosimilars that are not yet marketed (Planinc et al., 2017).

Our data also shows that reporting the QA types over time is likely influenced by the development of regulatory guidelines of biosimilars that were published by the EMA and US-FDA during the study period. This regulatory guidance effect is most evident for reporting of immunochemical activity that was not reported in several included publications of (intended) biosimilars containing antibodies before the publication of EMA guidance on biosimilars containing monoclonal antibodies in 2012 (EMA, 2012). The EMA updated guideline entitled *"Similar biological medicinal products containing biotechnology-derived proteins as active substance: quality issues",* and the US-FDA released a guideline entitled *"quality considerations in demonstrating biosimilarity of a therapeutic protein product to a reference product"* in 2014, and 2015, respectively (EMA, 2014; US-FDA, 2015a) Although both guidelines, especially compared to the first version of the EMA quality guideline, placed more emphasis on all QA types included in the classification scheme, an increase in reporting was only found for primary structure, HOSs and PTMs.

Presenting biosimilarity assessment of QAs in scientific publications is one of several strategies to improve learning in biosimilar development, and to maintain communications with the scientific and medical community. The development of biosimilars together with continuous advancement in science and analytical technology facilitates the understanding about the active biological substance by the regulators and medical community. In the past, reference companies actively stated that producing biosimilars was almost an impossible task due to structural and manufacturing process complexity of biological drugs (Blackstone and Joseph, 2013). Several analytical analyses of different batches of reference products have shown that there is always some batch-to-batch variability in QAs as a result of, among others, changes in the production process (Schiestl et al., 2011; Planinc et al., 2017; Kim et al., 2017). The availability of a complete assessment of QAs could result in better understanding of the role of QAs in establishing biosmilarity and comparability not only for biosimilars at approval time but also for the reference biologicals as well as biosimilars when changes in the manufacturing process after the regulatory approval are introduced. However, among several QAs of biologicals, only a subset of these are potentially relevant to efficacy, safety, and dosing of a drug, which are also known as critical quality attributes (CQAs). As such, CQAs must be routinely monitored and controlled to keep them within an appropriate limit, range, or distribution to assure the quality of a biological drug (ICH, 2009). A future challenge is to identify the CQAs and understand their relation to functional and clinical outcomes. This might result in a list of CQAs that matter most for establishing the biosimilarity, which could reshape the current regulatory requirements of biosimilars by reducing unnecessary comparative clinical trials currently required for licensing (Wolff-Holz et al., 2019).

To our knowledge, this review is the first study that identifies the QA types reported in biosimilarity assessments presented in full-text scientific publications and describes the dynamics of publishing biosimilarity assessments and reporting of QA types over time. We

constructed a classification scheme of QA types, based on regulatory guidelines and input from regulatory experts, to allow for a uniform assessment of the included publications. The study also sheds lights on how many biosimilars that were granted regulatory approval from the EMA and/or US-FDA have a published biosimilarity assessment of QAs to support the core evidence of biosimilarity in the literature.

Nevertheless, the present study has several limitations. First, a quality assessment for the included publications was not undertaken as there is no tool available to assess the strength/validity of the technical and analytical studies. Second, QAs might be missed due to heterogeneity in how these are defined between scientific publications, as no official classification system for QAs in biosimilarity assessments exists. However, we applied a classification scheme co-developed in collaboration with regulatory experts thus it is unlikely that QAs were missed. Third, it cannot be determined whether QAs not reported were actually not tested or tested but not published by the author(s) because the present study only relied on published data. Finally, certain publications might be not included due to different languages or being unable to pick up in our search strings. However, we developed a search strategy in which the reference lists of review papers were manually checked to identify publications potentially missing in the electronic search, which resulted in an insignificant number of additional publications relevant to the study objective.

5. Conclusions

We observed a clear increase in the number of scientific publications that present biosimilarity assessments of QAs for (intended) biosimilars over time, in line with an increased number of (intended) biosimilars for antibodies and hormones under development, with a large variability in the completeness of reporting QAs in these. Publishing of biosimilarity assessments and reporting of QA types over time appears to be affected by regulatory actions that occurred in 2012-2015, including the regulatory approval and the development of regulatory guidelines for biosimilars. Availability of a complete, publicly accessible (open access) and unbiased biosimilarity assessment of QAs, as part of a trusted and transparent regulatory process, will contribute to increased confidence and acceptance of biosimilars in clinical practice.

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Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Ali M. Alsamil: Conceptualization, Data curation, Funding acquisition, Validation, Formal analysis, Visualization, Writing - original draft, Methodology, Investigation, Writing - review & editing. **Thijs J. Giezen:** Conceptualization, Data curation, Funding acquisition, Validation, Methodology, Investigation, Writing - review & editing, Project administration, Resources, Supervision. **Toine C. Egberts:** Conceptualization, Data curation, Funding acquisition, Validation, Methodology, Investigation, Writing - review & editing, Project administration, Resources, Supervision. **Hubert G. Leufkens:** Conceptualization, Data curation, Funding acquisition, Validation, Methodology, Investigation, Project administration, Writing - review & editing, Resources, Supervision. **Arnold G. Vulto:** Methodology, Investigation, Writing - review & editing. **Martijn R. van der Plas:** Methodology, Investigation, Writing - review & editing. **Helga Gardarsdottir:** Conceptualization, Data curation, Funding acquisition, Validation, Methodology, Investigation, Writing - review & editing, Project administration, Resources, Supervision.

Declaration of Competing Interests

AMA, TJG, TCE, HGL, MRP and HG declare that they have no conflict of interest. AGV has an interest/relationship or affiliation in the form of: Consultant for AbbVie, Biogen Idec Ltd; Fresenius/Kabi, Pfizer / Hospira Inc; Sandoz/Novartis Ltd.; Samsung Bioepis, Speaker's Bureau Participant with Amgen Inc; Biogen Idec Ltd; Bristol Meyers Squibb; F. Hoffmann-La Roche Ltd; Eli Lilly; Febelgen/Medaxes; Medicines for Europe AISBL; Mundipharma; Pfizer/Hospira, and Hexal/Sandoz Ltd. Advisory Board for AbbVie; Accord Healthcare, Amgen Inc; Biogen Idec Ltd; Boehringer-Ingelheim; Pfizer / Hospira, and Sandoz/Hexal AG.

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Supplementary materials

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