Position paper

Bioequivalence of recombinant factor VIII products: a position paper from the Italian Association of Hemophilia Centers

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Over the last three decades, the continuous evolution of recombinant factor VIII (rFVIII) concentrates for replacement treatment of hemophilia A, including recent extended half-life products, implies that patients may switch from one product to another, technologically more advanced, with the aim of improving treatment efficacy, safety, management and, ultimately, quality of life. In this scenario, the issues of bioequivalence of rFVIII products and the clinical implications of their interchangeability are keenly debated, in particular when economic reasons or purchasing systems influence product availability and choices. Although sharing the same Anatomical Therapeutic Chemical (ATC) level, rFVIII concentrates, as other biological products, show relevant differences in terms of molecular structure, source and manufacturing process, which make them unique products, recognized as new active substances by regulatory agencies. Moreover, data from clinical trials with both standard and extended half-life products clearly document the large inter-patient variability of pharmacokinetic profiles after administering the same dose of the same product; in cross-over evaluations, even when mean values are comparable, some patients show better patterns with one product or with the comparator one. Pharmacokinetic assessment thus reflects the response to a specific product in the individual patient, with his genetic determinants, only partially identified, affecting the behavior of exogenous FVIII. These concepts, consistent with the currently recommended approach of personalization of prophylaxis, are discussed in this position paper endorsed by the Italian Association of Hemophilia Centers (AICE), highlighting that ATC or other available classifications do not completely consider differences between drugs and innovations and that substitutions of rFVIII products will not invariably ensure the previously achieved clinical outcomes or generate benefits for all patients.

Keywords: *bioequivalence, hemophilia, pharmacokinetics, recombinant FVIII concentrate.*

Arrived: 6 October 2022
Revision accepted: 12 December 2022
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INTRODUCTION

After the discovery of cryoprecipitate in 1964 by Judith Pool[1], lyophilized plasma-derived (pd) factor VIII (FVIII) concentrates became available in the early 1970s, marking the beginning of the modern era of treatment of hemophilia A[2]. Cloning of the *F8* gene

40 years ago and the subsequent development of recombinant FVIII (rFVIII) concentrates started a new exciting era of continuous progress of replacement treatment, first triggered by safety issues (first-, second- and third-generation rFVIII, progressively eliminating human/animal proteins in the manufacturing process), then by the need to improve bleeding protection and reduce treatment burden (the recent extended half-life [EHL] products)[3-6]. These achievements greatly facilitated the management and personalization of prophylaxis, the regimen of regular, long-term administrations of concentrate aimed at preventing bleeding and joint deterioration, strongly recommended in patients with severe FVIII deficiency (<1 IU/dL) or bleeding phenotype[7]. The high standard of care greatly enhanced the quality of life of hemophilia A patients in the last 30 years and their life expectancy reached that of males in the general population, at least in high-income countries[6].

The continuous evolution of rFVIII concentrates implies that patients may switch from one product to another, technologically more advanced, with the aim of improving treatment efficacy, safety, management, and ultimately the patient's quality of life. However, this issue is quite complex and involves both personalization of treatment, according to individual pharmacokinetics and clinical features or needs, and the molecular characteristics of the products which, although belonging to the same class of rFVIII concentrates, have substantial structural differences that significantly influence their pharmacokinetic and pharmacodynamic properties[8]. Although sharing the same Anatomical Therapeutic Chemical (ATC) level, rFVIII products differ greatly in terms of molecular structure, source materials, manufacturing process and safety and efficacy profiles of their active substance (**Table I**). Indeed, the issue of the bioequivalence of rFVIII products and their possible complete interchangeability in clinical use is currently being keenly debated, in part also because of the increasing impact in recent years of economic reasons or national/regional tender contract systems underlying switches in rFVIII products, beyond clinical needs or preferences of patients/parents[9]. On this background, the Italian Association of Hemophilia Centers (AICE) set up a multidisciplinary expert panel aimed at analyzing the available evidence from literature and regulatory documents about bioequivalence of the different licensed rFVIII. The data retrieved, the implications for therapeutic choices and drug availability, with reference to the Italian context, are discussed in this manuscript, endorsed by AICE as a position paper.

THE ISSUE OF BIOEQUIVALENCE

rFVIII concentrates, like other biological drugs, are complex molecules that undergo a sophisticated production process. The gene and the expression cells, as well the manufacturing procedures, define the characteristics of the drug; modifications in any part of the process can significantly alter the composition of the protein and, consequently, its effectiveness and safety. Furthermore, there may be a certain degree of variability between different batches of the same product[10].

To be perfectly alike, two drugs should have an identical composition of active ingredients and excipients, and be subjected to the same production process, in the same manufacturing plant. To exclude that differences between products do not determine significantly different therapeutic results, the products must be bioequivalent. Bioequivalence is, therefore, a pre-condition of equivalence. Two products are defined as bioequivalent if they contain the same quantity of the active ingredient, have the same pharmaceutical form (even with different excipients), have identical or comparable quality standards and the same route of administration, and if their bioavailability, after administration at the same doses, is so similar that they are unlikely to produce significant differences in efficacy and safety. Bioequivalence studies are used to demonstrate that differences in bioavailability do not exceed a certain range of variability, established by international convention, and deemed compatible with therapeutic equivalence[11].

"True" equivalence, therefore, only applies to identical active ingredients, (although with different excipients), that have in common dosage, route of administration and pharmaceutical form; as a consequence, their approved therapeutic indications are also bioequivalent.

In the case of drugs of both biological and non-biological origin defined as "complex" (**Table II**), the requirement of bioequivalence is not sufficient to ensure therapeutic equivalence. The more complex the

Blood Transfus 2023; 21: 441-451 doi: 10.2450/2023.0235-22

Table I - *Recombinant factor VIII concentrates available in Italy**

Product brand (company)	Recombinant protein	Cell line	Fractionation	Viral inactivation	Specific activity (IU/mg of total proteins)	Comments
A. Standard half-life						
Recombinate (Takeda, Tokyo, Japan)	Octocog alfa (full-length)	CHO	• mAb affinity chromatography • ion exchange chromatography	• None	>4,000	Albumin + VWF traces
Advate (Takeda)	Octocog alfa (full-length)	CHO	• mAb affinity chromatography • ion exchange chromatography	• Solvent/detergent (TNBP/Triton X-100/ Polysorbate 80)	4,000-10,000	Protein free
ReFacto AF ReFactoAF fuseNGO (Pfizer, New York, NY, USA)	Moroctocog alfa (B-domain deleted)	CHO	• Ion exchange chromatography • hydrophobic interaction chromatography • size exclusion chromatography affinity • chromatography (synthetic peptide, 27 amino acids)	• Solvent/detergent (TNBP/Triton X-100) • nanofiltration (35 nm pore size filter)	7,600-13,800	Protein free
NovoEight (Novo Nordisk, Bagsværd, Denmark)	Turoctog alfa (B-domain truncated)	CHO	• mAb affinity chromatography • ion exchange chromatography • size exclusion chromatography	• Detergent (Triton X 100) • nanofiltration (20 nm pore size filter)	8,300	Protein free
Nuwiq (Octapharma, Lachen, Switzerland)	Simoctocog alfa (B-domain deleted)	HEK	• Ion exchange chromatography • affinity chromatography • size exclusion chromatography	• Solvent/detergent (TNBP/Triton X-100) • nanofiltration (20 nm pore size filter)	9,500	Protein free
Kovaltry (Bayer, Leverkusen, Germany)	Octocog alfa (full-length)	BHK	• Ion exchange chromatography • mAb affinity chromatography • metal chelate affinity chromatography	• Detergent (Triton X 100) • nanofiltration (20 nm pore size filter)	4,000	HSP70 gene Protein free
Afstyla (CSL Behring, King of Prussia, PA, USA)	Lonoctocog alfa (single-chain)	CHO	• mAb affinity chromatography • ion exchange chromatography (4 steps) • size exclusion chromatography	• Solvent/detergent (TNBP/Polysorbate 80) • nanofiltration (19 nm pore size filter)	16,000	Protein free
B. Extended half-life						
Elocta (Sobi, Stockholm, Sweden)	Efmoroctocg alfa (B-domain deleted)	HEK	• Centrifugation • ion exchange chromatography • hydrophobic interaction chromatography • size exclusion chromatography	• Detergent (Triton X-100) • nanofiltration (15 nm pore size filter)	4,000-10,000	IGg1 Fc-fusion protein
Adynovi (Takeda)	Ruricotocog alfa pegol (full-length)	CHO	• mAb affinity chromatography • ion exchange chromatography	• Solvent/detergent (TNBP/Triton X -100/ Polysorbate 80)	4,000-6,500	Random PEGylation 20 kDa
Jivi (Bayer)	Damoctocog alfa pegol (B-domain deleted)	BHK	• Anion exchange chromatography • mAb affinity chromatography cationic exchange chromatography • size exclusion chromatography	• Detergent (Triton X 100) • nanofiltration (20 nm pore size filter)	10,000	Site-specific PEGylation 60 kDa, branched
Esperoct (Novo Nordisk)	Turoctocog alfa pegol (B-domain truncated)	CHO	• mAb affinity chromatography • anion exchange chromatography • size exclusion chromatography	• Detergent (Triton X 100) • nanofiltration (20 nm pore size filter)	9,500	Site-specific glycoPEGylation 40 kDa, branched

Recombinant factor VIII products are listed in order of time of approval and market availability. BHK: baby hamster kidney; CHO; chinese hamster ovary; HEK: human embryonic kidney; HSP70: human shock protein 70; IgG1Fc: crystallizable fragment of immunoglobulins G1; mAb: monoclonal antibodies; TNBP: tri-*n*-butyl-phosphate; VWF: von Willebrand factor.

Table II - *Examples of complex drugs*

Category	Example
Complex active ingredients	Peptides, polymeric compounds, complex mixtures of active ingredients, naturally derived components
Complex formulations	Liposomes and colloids
Complex routes of administration	Drugs acting locally such as dermatological products and complex ophthalmological products, otological dosages in the form of suspensions, emulsions, or gels
Complex dosages	Transdermal, metered-dose inhalers and sustained-release injectables
Complex drug-device combinations	Auto-injectors, inhalers with dispenser
Others	Other products where complexity or uncertainty related to the approval path or possible alternative approach would benefit from early scientific engagement

pharmacological structure, the more challenging the definition of therapeutic equivalence[12]. Therefore, the complex structure of FVIII complicates the definition of therapeutic equivalence among rFVIII products.

PHARMACOKINETIC EQUIVALENCE OF RECOMBINANT FACTOR VIII CONCENTRATES

Pharmacokinetic (PK) profiles of factor concentrates are a surrogate of the products' clinical efficacy because PK parameters, such as the area under the curve (AUC), clearance, half-life (HL), and the lowest pre-infusion level (trough), provide only some information about the effect of replacement therapy on the risk of bleeding of each patient[13]. It is commonly agreed that the prophylaxis regimen should be tailored according to the individual PK profile[7]; however, many other individual and external factors contribute to the annual bleeding rate, the most frequently reported efficacy outcome. The bleeding phenotype of each hemophilia patient results from his/her specific genotypic profile, including the *F8* variant but also other genetic determinants that can affect the behavior of endogenous and exogenous administered FVIII, and from non-genetic (behavioral, musculoskeletal) factors[7]. In other words, each hemophilia patient represents a unique biological entity. The response to the infusion of the same dose of the same product varies greatly among hemophilia A patients enrolled in phase I/II PK studies of all FVIII concentrates. Personalized prophylaxis allows replacement treatment to be customized to the needs of each patient, avoiding unnecessarily high, not cost-efficient, FVIII levels or, conversely, unsafe low levels. The assessment of bioequivalence of rFVIII concentrates should consider the intrinsic characteristics of each product, making comparisons within each product class, standard half-life (SHL) and EHL rFVIII.

Standard half-life recombinant factor VIII concentrates

The data from a large population of patients (100 adults and 52 adolescents) given a single dose of octocog alfa (50 IU/kg), showed an *in vivo* recovery (IVR) ranging from 1 to 5 IU/dL/IU/kg[14]. As IVR is used to define the loading dose, it is evident that the same dose does not have the same effect in all patients, i.e., "one size doesn't fit all". To achieve the same post-infusion highest concentration (Cmax), the loading dose should be reduced in patients with a higher IVR and increased in those with a lower IVR. As an example, considering the extremes of the IVR range14, the same Cmax 60 IU/dL will require a loading dose of 60 IU/kg and 12 IU/kg in two patients with an IVR of 1 IU/dL/IU/kg and 5 IU/dL/IU/Kg, respectively.

The slope of the decay curve after the Cmax also shows large inter-patient variability in all PK studies; for example, in the NuPreviq (NCT01863758) study[15], the HL of simoctocog alfa in 66 patients ranged from 6 to 31 h, the mean being 15.1±4.7 h. However, the intra-patient variability of PK is smaller than inter-patient variability, therefore dosing can and must be tailored, especially for repeated infusions, as during prophylaxis, through individual parameters of PK[13]: the Cmax, alfa HL (the rate of decline in plasma concentrations due to the drug redistribution from the central to the peripheral compartment), the beta HL (the rate of drug decline due to its elimination by metabolism) and, above all, clearance.

The inter-patient variability was clearly shown in the cross-over regulatory (phase I/II) studies, requested by the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) for the evaluation of the bioequivalence of some new SHL FVIII concentrates, compared to previously available ones[16,17]. Bioequivalence is generally evaluated by the mean values of PK parameters, mainly HL. Although mean values are not significantly different, individual patients' data reveal different PK patterns: some patients show a better profile with one concentrate while others have more favorable parameters when receiving the comparator

product. These discrepancies among individual PK parameters (IVR, AUC) were observed in two cross-over studies, one comparing turoctocog alfa *vs* octocog alfa protein-free (PF) produced using Chinese hamster ovary (CHO) cells[16] and another comparing a pdFVIII *vs* a SHL rFVIII concentrate[17]. Nevertheless, in 18 previously treated patients with severe hemophilia A enrolled in a cross-over, open-label, PK study comparing octocog alfa PF derived from baby hamster kidney (BHK) cells against octocog alfa PF derived from CHO[18] the AUC resulted higher (1,660 *vs* 1,310 IU*h/dL) and the HL longer (14.5 *vs* 11.7 h) with the first product. According to the simulations performed using a population PK tool (NONMEM®, ICON, Hanover, MD, USA), the median time to reach the trough level of 1 IU/dL after a dose of 25-50 IU/kg was 27% longer for BHK cell-derived octocog alfa PF, which maintained plasma FVIII levels >1 IU/dL with 14.4 IU/kg compared to 39 IU/kg of the other product.

Extended half-life recombinant factor VIII concentrates

All EHL rFVIII concentrates have been compared with previous SHL rFVIII products in head-to-head studies, to show the HL improvement of the newly modified molecules, according to the licensing procedures of the FDA or the EMA. The cross-over studies of the new EHL rFVIII products showed an increase of HL in the range of 1-4 h, at variance with the outstanding increase of about 30-60 h shown in studies with the EHL recombinant factor IX concentrates[19]. Nevertheless, the new EHL rFVIII concentrates allow a reduction of the dosing frequency of prophylaxis from twice a week to every 5 days or more. A comparative evaluation of the bioequivalence of rFVIII EHL products is very difficult because the cohorts of patients enrolled in independent studies were different, as were the methods of the FVIII assay and PK sampling protocols.

Only head-to-head cross-over studies can provide information on differences between the EHL rFVIII concentrates. Very few comparative studies have been conducted, probably because of the low attraction of such issues for pharmaceutical companies. Furthermore, the comparison between different EHL rFVIII concentrates is not mandatory for the pre-licensure trials.

A PK study was done in Canada in 25 adolescent patients (12.1-18.4 years old), on the occasion of switching from efmoroctocog alfa to rurioctocog alfa pegol[20]. The mean terminal HL, evaluated using the WAPPS-Hemo PK tool[21], was comparable (10.4-23.4 *vs* 11.0-23.6 h for efmoroctocog alfa and rurioctocog alfa pegol, respectively) when FVIII levels were measured by a one-stage clotting assay, but slightly longer for efmoroctocog alfa (12.0-25.5 *vs* 10.3-22.9 h), when a chromogenic assay was used. No significant differences were observed between the two concentrates concerning the AUC, volume at the steady state, and the time to reach the trough levels of 5, 3 and 1 IU/dL. However, individual data showed that about half of patients had better PK properties with one product or with the other one[20].

A single-center, randomized, cross-over PK study compared damoctocog alfa pegol and efmoroctocog alfa in a cohort of 17 (one outlier excluded) previously treated patients with severe hemophilia A, aged 18-65 years[22]. After a loading dose of 60 IU/kg of both concentrates, seven blood samples during the first 24 h and four every day up to 120 h were collected for FVIII measurements (one-stage clotting assay). Individual PK was studied using non-compartmental analysis. The Cmax and IVR of efmoroctocog alfa were higher than those of damoctocog alfa pegol (194 *vs* 150 IU/dL, p<0.05: 3.09 *vs* 2.26 IU/dL/IU/kg, p<0.05, respectively), whereas the latter showed a greater AUC, longer mean residence time and longer HL (3.010 *vs* 2.400 IU*h/dL, p<0.0001; 23.2 *vs* 19.9 h, p<0,001; 16.3 *vs* 15 h p<0.05, respectively) and smaller clearance and volume at steady state (0.020 *vs* 0.025 dL/h/kg, p<0.0001; 0.462 *vs* 0.497 dL/kg, p=0.06). According to a population PK model using NONMEM, the simulated median times to reach the troughs of 10, 5, 3, and 1 IU/dL were longer (10.9-13 h) in patients receiving damoctocog alfa pegol than in those receiving efmoroctocog alfa[22]. A similar cross-over PK study compared damoctocog alfa pegol *vs* rurioctocog alfa pegol in 18 adult patients, after a loading dose of 54.3 IU/kg and 61.4 IU/kg, respectively[23]. The AUC normalized for dose was 43.8 and 36.0 h*kg/dL, respectively. Damoctocog showed a reduced clearance compared to rurioctocog (1.65 *vs* 2.01 dL/h, respectively) and a longer HL (17.0 *vs* 16.0 h. respectively).

In the absence of head-to-head studies comparing clinical outcomes of EHL products, a new statistical tool, the matching adjusted indirect comparison (MAIC)[24], was used to evaluate the outcomes (annual bleeding rate and proportion of patients with zero bleeds) of the A-LONG phase III and PROTECT VIII phase II/III trials, which

enrolled 117 and 110 patients treated with efmoroctocog alfa and damoctocog alfa pegol, respectively. The mean annual bleeding rate of patients on efmoroctocog alfa was lower than that of patients on damoctocog alfa pegol (3.9 *vs* 4.9), while the proportion of patients with zero bleeds (46.5 *vs* 38.2%, respectively) was not statistically different.

More relevant differences in bioequivalence have recently been reported with the new fusion protein BIVV001 (rFVIIIFc-VWF-XTEN)[25]. A phase I/IIa trial enrolled 16 patients with severe hemophilia A, aged 18-65 years, who were given two single doses (25 IU/kg or 65 IU/kg) in consecutive PK studies comparing octocog alfa against BIVV001[26]. The mean HL of BIVV001 was 3-4 times longer than that of the comparator rFVIII (37.6 *vs* 9.1 h and 42.5 *vs* 13.2 h in the lower-dose and in the higher-dose group, respectively). BIVV001 is not yet licensed in the USA and Europe; confirmation of its clinical efficacy is expected from the results of the recently concluded phase III study. As the Committee for Medicinal Products for Human use (CHMP) of EMA pointed out[27], all rFVIII concentrates must be considered as new active substances (NAS), each different from the other. Moreover, each patient is different from the others due to the individual genotypic profile. *F8* genotype seems to have only small effects on the PK of pdFVIII as well as rFVIII concentrates. PK parameters such as alfa and beta HL, clearance and mean residence time of hemophilia A patients positive or negative for intron 22 inversion do not differ significantly (Morfini, *personal communication*). However, other genetic factors can affect the decay of infused FVIII concentrates. Due to the cross-reactivity of agglutinins, anti-A and anti-B, with von Willebrand factor (VWF), patients with blood group O have a lower basal level of VWF and faster decay of FVIII concentrates[28-32]. Patients with the low-density lipoprotein-receptor c.81 (exon 2) TT genotype showed a shorter alfa HL and faster clearance than those with the CC genotype[32]. With regard to asialoglycoprotein receptor 2 (*ASGR2*) genotypes, c.95TT homozygous patients showed longer alfa HL, while shorter beta HL and mean residence time were detected in c.95TC heterozygotes[33]. C-type lectin-domain family 4 member M (CLEC4M) is the receptor of VWF[34] and through this, together with blood group O, can affect FVIII turnover[35,36]. The role of these and other extra-*F8* gene polymorphisms in FVIII pharmacokinetics has been reviewed recently[36,37].

Laboratory monitoring of different recombinant factor VIII products

Clotting factor concentrates are produced in licensed facilities and each lot is assayed for clotting factor activity expressed by a specific potency. This lot potency designation is needed for dosage calculations and is used by the prescribing physician in therapeutic decisions about the prophylaxis regimens and the management of bleeding episodes and surgical interventions. Hence, reliable methods for correct and precise quantitation of FVIII in therapeutic concentrates are essential. Presently, the one-stage clotting assay (based on the activated partial thromboplastin time) and the chromogenic assay (based on the formation of tenase complex) are widely used for the evaluation of FVIII potency. Substantial discrepancies between FVIII assays have been reported in the different pharmacodynamic and PK studies of rFVIII concentrates. These discrepancies may in part be caused by differences in reference plasmas, reagents, procedures, the recipients and, potentially, by the nature of the rFVIII products themselves[38]. Notably, the differences in specific activities of rFVIII products provided by manufacturers closely reflect the differences observed for rFVIII mass content in those products. Different pdFVIII and rFVIII products were tested at an equimolar FVIII protein concentration in a thrombin generation assay[39]. In that study, the molar FVIII protein, designated by each manufacturer as having 1 U/mL potency, varied from 0.38 nM to 1.1 nM in the tested products[39]. Hence, these results showed that infusions of equal FVIII potency, based on the manufacturers' assessment, involve the administration of different amounts of FVIII protein. Whether these discrepancies affect the efficacy of treatment is unclear, however, these findings implicitly outline the lack of bioequivalence of different rFVIII products.

(BIO)EQUIVALENCE AND THE ATC CLASSIFICATION

The ATC system was specifically developed to classify the active ingredients of pharmaceutical preparations according to the organ or system on which they act and their therapeutic, pharmacological and chemical properties. A seven-digit code, based on five hierarchical levels is assigned (**Table III**). However, the ATC system does not necessarily reflect the recommended therapeutic use of drugs. In many main ATC groups, drugs with different therapeutic uses have been assigned to the

Table III - *The Anatomical Therapeutic Chemical classification of drugs*

Hierarchical levels	Digit(s) and meaning
Level I	A letter of the alphabet indicating the main **anatomical group**, i.e. the anatomical site of action of the drug*
Level II	Two numbers indicating the main **therapeutic group** to which the drug belongs
Level III	A letter of the alphabet indicating the **pharmacological therapeutic subgroup**, according to the different mechanisms/site of action
Level IV	A letter of the alphabet indicating the **chemical subgroup** in the frame of the pharmacological therapeutic subgroup
Level V	A two-digit number referred to the specific **chemical** substance

ATC: Anatomical Therapeutical Chemical.
*Main anatomical groups are 14: A) Gastrointestinal system and metabolism; B) Blood and hematopoietic organs; C) Cardiovascular system; D) Dermatological drugs; G) Genitourinary system and sex hormones; H) Systemic hormonal preparations, excluding sex hormones and insulin; J) General antimicrobials for systemic use; L) Antineoplastic and immunomodulatory drugs; M) Musculoskeletal system; N) Nervous system; P) Insecticidal and repellent antiparasitic drugs; R) Respiratory system; S) Sense organs; V) Various.

second, third and fourth levels, without specifying the main indication. Many medicines are used and approved for two or more indications, but usually only one ATC code is assigned. Additionally, ATC codes are often assigned based on mechanism of action rather than therapeutic use; therefore, an ATC group can include medicines with many different indications, or drugs with a similar therapeutic use can be classified into different ATC groups. Moreover, drugs, such as coagulation FVIII and factor IX concentrates, have the same indications (treatment of hemophilia A/B) and mechanism of action (replacement of congenital factor deficiency) and share the same ATC code, but are characterized by different complexity, production methods and pharmacokinetic profile, currently not reflected by the ATC classification. Such an inconsistency limits the main objective for which the ATC was developed, i.e. addressing drug utilization studies. The ATC code enables information to be obtained on the entire therapeutic category "Factors VIII/IX", but not on the use of individual products. This problem has been overcome by the introduction of more structured drug classifications, such as the Generic Product Identifier in the USA[40].

The example of factor concentrates also highlights an intrinsic limit of the current ATC classification, i.e. that of not taking into account the evolution of biotechnologies. Indeed, apart from innovations concerning indication of use and mechanism of action, leading to a new code in the first ATC levels, most innovative drugs are the evolution of active ingredients already codified, and are aimed at improving the administration, handling, PK, functionality and clinical performance of the molecules. Therefore, the ATC code may not be able to keep track of these changes and new products, licensed by regulatory organizations and deemed worthy of being reimbursed, cannot receive specific ATC codification and recognition for drug utilization studies.

Overall, the ATC classification, despite being constantly updated (two sessions yearly), is not adequate to follow pharmaceutical innovations completely and dynamically and to address the problem of drug (bio)equivalence. These limitations may have implications in the concrete terrain of the purchase of drugs, as in the Italian context. Although the ATC classification is not used for regulatory and pricing purposes by the Italian authorities, nor for marketing purposes by manufacturers, it is a practical and fast system for classifying drugs, useful in the purchasing phase, but not exhaustive and precise in differentiating drugs. On the whole, the rules of the purchasing system cannot solve the problem and flatten all the differences between similar products. Therefore, in the absence of unequivocal parameters to differentiate the products, and with the misunderstanding that products with the same ATC at the fifth level are "equal", the principle of the lowest price is simply applied.

As reported in the second Position Paper on biosimilar drugs drawn up by the Italian Agency of Drugs (AIFA)[41], medicinal products of biological origin, such as FVIII concentrates, are obtained "through procedures that operate on living systems (animal cells), with numerous aspects of heterogeneity linked to the host cell and the transfected plasmid used to induce the expression of the desired protein, as well as the growth and fermentation conditions and the purification methods". Moreover, AIFA clearly states that the production process introduces elements of differentiation, helping to determine the

uniqueness of a product. The production process of these drugs is so distinctive that it can be said that "the product is the production process". Therefore, each biological product represents a unique product and significant differences may exist even between biological originators and their biosimilars. In this respect, it is questionable for a contracting authority to consider "comparable" biological drugs, such as FVIII concentrates, which are "unique" by their own nature, and clearly different for active molecules (full-length, B-domain deleted or truncated, single-chain) and PK properties. Moreover, in a national system in which each sector of the drug supply chain has its own rules (and timing) of analysis, the ATC classification cannot be used without knowing and taking into account its limits.

EQUIVALENCE IN THE EUROPEAN AND ITALIAN REGULATORY CONTEXT

Definitions of the terms "drug class" and "class effect" are not easy to find in the scientific literature or in established regulations. According to the European Academy of Patents, a "class effect refers to similar outcomes, therapeutic effects and adverse effects of two or more drugs. All products within a class are presumed to be closely related according to three concepts: chemical structure, mechanism of action and pharmacological effects". Thus, drugs with similar chemical structure, mechanism of action, or pharmacological effects can result in a "class effect".

Excluding the case of drugs with expired patents, drugs of the same class are certainly not "interchangeable", as each agent has a unique PK profile, which can make one more suitable than another for a specific treatment regimen or group of patients. Substitution between different drugs of a class can lead to adverse effects or lack of efficacy, if only the "class" effect is considered.

In the so-called Equivalence Guidelines (decision. 818/2018)[42] AIFA states that "the evaluation of therapeutic equivalence is a method through which it is possible to compare medicines containing different active ingredients in order to identify, for the same indications, areas of therapeutic superimposition, in which, in the light of scientific knowledge, clinically relevant differences in terms of efficacy and safety are not found". In practice, AIFA's position outlines the concept that if "there are peculiarities between the individual active ingredients, they must be identified and brought into clinical practice". In identifying equivalence criteria -which AIFA defines on a specific form at the request of the Regions over a period of 90 days- it is established that drugs with the following requisites may be admitted to an evaluation of therapeutic equivalence: (i) they are active ingredients for which there is at least 12 months experience of use (reimbursable by the national health system); (ii) there is evidence of efficacy from studies that do not allow the demonstration of superiority between drugs or from head-to-head studies that do not include hypotheses of superiority; (iii) they belong to the same level IV of the ATC classification; (iv) they have overlapping main therapeutic indications (section 4.1 of the Summary of Product Characteristics); (v) they are administered by the same route; and (vi) they have dosage schedules that enable a therapeutic intervention of overlapping intensity and duration.

Drugs that have been shown to be superior to a clinical value deemed significant by the AIFA Technical Scientific Committee (CTS) by means of phase III and IV randomized controlled trials, systematic reviews or European Public Assessment Reports (EPAR), and by observational comparative studies for safety, are excluded from the equivalence assessment.

This document also considers the possibility of carrying out assessments of specific situations not envisaged in the previous points. Finally, AIFA explicitly states that the declaration of equivalence is linked to the centralized competing purchases of drugs with unexpired patents and therefore, to support the Regions in saving pharmaceutical expenditure[42].

EMA and AIFA pronouncements on recombinant factor VIII concentrates

The European and Italian regulatory organisms, EMA and the AIFA, produced some documents in recent years in order to establish whether the different formulations attributable to the rFVIII products are equivalent[27,43-46].

Starting from the recognition that rFVIII concentrates are biological drugs, it must be determined whether the commercially available products can be traced back to the same active substances.

An active substance is defined in article 1 of Directive 2001/83/EC as "any substance or mixture of substances intended to be used in the manufacturing of a medicinal

product and, when used in its production, becomes an active ingredient of that product intended to exert a pharmacological, immunological or metabolic action with a view to restoring, correcting or modifying physiological functions or to make a medical diagnosis"[45].

A NAS includes a biological substance not previously authorized in a medicinal product for human use or a biological substance previously authorized but differing significantly in properties with regard to safety and/or efficacy, which is due to differences in one or a combination of the following: molecular structure, nature of the source material or manufacturing process. The CHMP, after reviewing the characteristics of the substance, defines whether it is a "NAS" during the authorization process.

The regional Administrative Tribunal of Piedmont by order n. 833/2018 (23.05.2018) asked the EMA to confirm whether the active substances contained in all rFVIII concentrates can be considered as the same active substance. The EMA, based on the requirements of a drug to be defined as a new substance, declared that turoctocog alfa, simoctocog alfa and lonoctocog alfa do not contain the same active substance and they are considered NAS[27]. Octocog alfa PF, derived from either BHK or CHO cells, and moroctocog alfa are not NAS[27]. In a subsequent response to a further specific request from the Administrative Court of Piedmont, EMA reiterated that lonoctocog alfa and octocog alfa from BHK cells do not have the same active substance[43]. Moreover, AIFA, again following a request from the Administrative Court of the Piedmont Region, took a position on the question of whether all rFVIII concentrates produced without the addition of any exogenous human and animal protein in the entire production process, including the final formulation with an EHL, "have the same active substance"[44]. By adopting the above principles of the EMA, AIFA highlights that EHL rFVIII products are biotechnological products, the technologies used are distinctive (fusion with different long HL plasma proteins, such as albumin or the Fc fragment of immunoglobulins, different approaches of PEGylation) and, while containing the same molecular fraction responsible for the pharmacological activity (active moiety), they are not attributable to the same active substance. Therefore, in the case of rFVIII concentrates, the association within the same ATC class V does not automatically mean that they can be considered bioequivalent[46].

The active ingredients contained in the various rFVIII concentrates had different names on the basis of the International Nonproprietary Name (INN) and, during the authorization process, obtained the status of NAS.

Finally, in the same document, AIFA recalls the requirements that should be fulfilled in order to define active ingredients that, albeit different, are equivalent from a therapeutic point of view, as reported above[45]. These characteristics are not present in all EHL FVIII products.

CONCLUSIONS AND PERSPECTIVES

In conclusion, the SHL and EHL rFVIII concentrates currently available are characterized by wide heterogeneity and complexity. The different manufacturing procedures for each product and the different genetic characteristics of each patient affect the clinical response and make it impossible to standardize them as a unique replacement therapy for all hemophilia A patients.

The selection of the most appropriate concentrate and the safer dose/regimen must be defined according to the individual PK of each patient. With this approach, it is possible to tailor treatment according to the individual clinical characteristics and needs, with the aim of providing protection from bleeding, preserving joint health and improving the quality of life. However, regular and careful clinical and laboratory follow-up of patients is needed in order to verify whether treatment prescribed on the basis of the individual PK fulfils these objectives, thus achieving personalized regimens, adjusted according to the bleeding rates, possible changes in lifestyle or comorbidities, and optimizing clinical outcomes in each patient[7].

The analysis of the ATC classification suggests that, in its aim of systematization, it has intrinsic rigidities that ultimately limit its ability to completely reflect differences between drugs and consider innovations. Therefore, the question of the equivalence between drugs belonging to the same ATC group remains unresolved and not codified. The first possible and necessary solution is acceleration of the entire path of defining a new ATC, to match the speed of pharmaceutical-technological innovations that arrive in clinical practice. In this respect, more frequent (at least quarterly) sessions of the new ATC and amendments would be helpful.

Furthermore, as the ATC classification was developed to be used in the post-licensure phase of the life of drugs, in clinical practice, the status of "equivalent" and "bioequivalent" should be acknowledged by the World Health Organization, during the modification phase, exactly as previously assessed in the regulatory phase for each drug. In this way, all situations in which "similar" drugs are not (bio)equivalent could also be settled by exclusion. This important procedural change could help to overcome "sterile" coding, unable to follow fast and complex biotechnological innovations.

Finally, it should also be kept in mind that not all SHL and EHL rFVIII fulfil the EMA and AIFA requirements for therapeutic equivalence, so substitution of one rFVIII product by another will not invariably ensure the previously achieved clinical outcomes or generate a benefit for all patients, thus challenging therapeutic efficacy and safety and quality of life.

Costs for hemophilia treatment are undoubtedly huge for public health systems. Tender approaches have been shown to reduce this impact, but limit the accessibility to some products for all or proportions of patients, irrespective of clinical needs. Legislation on this issue is currently dynamic and heterogeneous in different countries and regions, such as Italy. In some cases, a reasonable common price per unit of FVIII for all concentrates has been proposed to pharmaceutical companies to achieve product market access. This or other strategies, to be shared by health authorities and hemophilia treaters, supported by their scientific societies, and patient associations seem useful for both improving the affordability of treatment and guaranteeing therapeutic choices based on individual PK and clinical characteristics, also providing better cost-effectiveness and cost-utility of treatment by optimizing patients' bleeding, joint protection and quality of life.

AUTHORS' CONTRIBUTIONS

The paper was conceived and written with the endorsement of the Italian Association of Hemophilia Centers (AICE). EZ, RDC, MF, MM and GP were members of the multidisciplinary working group who defined the issues to be addressed, analyzed the literature data and regulatory documents and wrote the first draft of the paper. The members of the AICE Council (AR, ACM, RCS, CS and AC) critically reviewed the final version of the paper, which was drafted by AC and AR and approved by all the Authors.

DISCLOSURE OF CONFLICTS OF INTEREST

EZ has received fees as a member of an advisory board for Bayer and Biomarin. RDC has received fees as a speaker for Bayer, Sanofi, Roche, Takeda and Sobi and as a member of a scientific advisory board for Bayer, Sanofi, Pfizer, Werfen, Sobi and Kedrion. MF has acted as a paid consultant or speaker for Bayer and Novo Nordisk. MM has acted as a paid consultant for Kedrion, Pfizer and Sanofi. GP has acted as a paid consultant/advisor/speaker for Takeda, UCB, Novartis, NOF and Medac and as a member of a scientific advisory board for Takeda. ACM has acted as an advisor for Bayer, CSL Behring, Roche and Sobi and received speaker fees from Bayer, CSL Behring, Kedrion, Novo Nordisk, Pfizer, Roche, Sobi and Takeda. CS has acted as a paid consultant/advisor/speaker for Bayer, Biomarin, CSL Behring, Novo Nordisk, Roche, Sobi and Takeda. RCS has acted as a paid consultant/advisor/speaker for Bayer, CSL Behring, Takeda, Novo Nordisk, Pfizer, Biomarin, Roche and Sobi. AC has acted as a paid consultant or speaker for Bayer, Kedrion, Novo Nordisk, Roche and Sobi. AR has acted as a paid consultant/advisor/speaker for Bayer, CSL Behring, Kedrion, Novo Nordisk, Pfizer, Roche, Shire/Takeda and Sobi.

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