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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

Evaluation of prophylactic polyclonal anti-D antibodies: Differences in Fc-glycosylation in commercial products

We are writing to share our efforts to help patients in preventing RhD Disease, an alloimmune condition also known as Haemolytic Disease of the Foetus and the Newborn (HDFN) [1]. To prevent a pathogenic immune reaction, an RhD negative mother carrying an RhD positive foetus should receive hyperimmune polyclonal RhD-specific IgG antibodies [1]. Monoclonal anti-D IgG have been produced by a variety of methods that give rise to differences in anti-D Immunoglobulin activity and some of these differences can be attributed to the glycans linked to the Fc region of IgG anti-D [2]. Glycomics is a rapidly developing discipline with the aim of identifying a relationship between glycan structures and protein functionality. In particular, the glycosylation of immunoglobulins is extensively studied due to the important role these proteins play in the immune response [3]. Previously published work [4, 5] has shown that anti-D products with low fucose (low fucosylation) and high galactose (high galactosylation) content may be more potent and protective for prophylaxis in HDFN. We decided to investigate the glycosylation pattern of two prophylactic anti-D immunoglobulin products,

TABLE 1 % Fucosylation, sialylation and galactosylation content of IMMUNORHO®, RhoGam® and IgVena®

	IMMUNORHO			RhoGam			IgVena		
Glycan structure	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Fucosylation (%)	83.31	81.55	79.43	81.19	79.74	77.00	95.04	95.41	95.36
Mean (%)		81.43			79.31			95.27	
CV (%)		2.38			2.68			0.21	
Sialylation (%)	24.96	25.70	25.53	26.03	27.22	21.26	17.77	18.90	20.93
Mean (%)		25.40			24.84			19.20	
CV (%)		1.53			12.68			8.33	
Galactosylation (%)	87.11	89.31	91.20	89.78	91.32	89.05	74.09	74.04	74.52
Mean (%)		89.21			90.05			74.22	
CV (%)		2.29			1.28			0.35	

TABLE 2 Breakdown of galactosyl content of IMMUNORHO®, RhoGam® and IgVena®

	IMMUNORHO			RhoGam			IgVena		
Glycan structure	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Agalactosyl (G0) (%)	12.90	10.67	8.79	10.22	8.69	10.95	25.91	25.96	25.47
Mean (%)		10.79			9.95			25.78	
CV (%)		19.08			11.55			1.05	
Monogalactosyl (G1) (%)	34.02	33.03	33.98	32.59	31.53	36.59	41.33	40.53	39.49
Mean (%)		33.68			33.57			40.45	
CV (%)		1.66			7.96			2.28	
Digalactosyl (G2) (%)	53.08	56.29	57.23	57.20	59.77	52.46	32.77	33.51	35.04
Mean (%)		55.54			56.48			33.77	
CV (%)		3.92			6.57			3.44	

IMMUNORHO® and RhoGam®, along with the intravenous immunoglobulin (IVIG) product IgVena®.

European Pharmacopoeia (Ph Eur) methods 2.7.13 B and C were used to determine anti-D potency for three lots of each anti-D product. For glycan analysis, anti-D products were affinity purified on group O, R_2 R_2 cells and further purified on immobilised protein G prior to preparing all samples (six lots of anti-D and three lots of IVIG) for Mass Spectrometry analysis using a GlycoWorks RapiFluor MS kit (Waters, UK). Glycan separation was carried out on an Acquity UPLC H-class Bio system (Waters, UK) with a BEH Glycan Amide column (Waters, UK) using in-house methodology. Data were acquired and processed manually using Empower 3.1 software. Peaks were assigned to glycan structures and each glycan structure was expressed as a percentage relative peak area of the total percentage area of assigned peaks.

All six batches of prophylactic anti-D complied with the Ph Eur specification for potency. There are clear differences in the mixture and abundance of glycan structures for anti-D and IVIG. In IVIG, fucosylated structures are typically the most abundant glycan forms (Table 1). Digalactosyl structures are in greater abundance in the anti-D products (Table 2) and in addition to low fucosylation [4, 5] important for enhanced ADCC activity. As reported for Rhophylac® [4, 5] and RhoGam [5] our results show that higher levels of sialylation and galactosylation and lower levels of fucosylation are present in IMMUNORHO and RhoGam products compared to IVIG. Further work is required to elucidate the link between glycosylation and anti-D immunoglobulin function. We intend to collect additional data to contribute to the better understanding of the properties of anti-D immunoglobulins in relation to the variation in IgG-Fc glycosylation profiles.

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CONFLICT OF INTEREST

F.M., A.S., E.A. and R.D. work full time for Kedrion Biopharma Inc. B.F. works full time for NIBSC.

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DATA AVAILABILITY STATEMENT

Data will be stored at Kedrion S.p.A. in the Global Medical Affairs Department.

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