

Seroprevalence of pre-existing NABs against AAV1, 2, 5, 6 and 8 in the South African Hemophilia B patient population

Anna Majowicz¹, **Nolukholo Ncete**², Floris van Waes¹, Sander J. van Deventer¹, Johnny N. Mahlangu², Valerie Ferreira¹
¹uniQure B.V., Amsterdam, The Netherlands. ²Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand, Faculty of Health Sciences and NHLS, 7 York Road, Parktown, 2193 Johannesburg, South Africa. **Poster No. P116**

BACKGROUND

The impact of pre-existing anti-AAV neutralizing antibodies (NABs) on liver transduction following systemic administration of AAV-based gene therapy varies between AAV serotypes. Titers of anti-AAV2 or anti-AAV8 NABs as low as 5 have been related to a decrease or even total impairment of AAV liver transduction after systemic delivery in humans [1, 2]. However, successful gene transfer has been reported in patients with anti-AAV5 NABs titers up to 340 and in non-human primates with titers up to 1030 [3]. A significant proportion of individuals develop humoral immunity against various AAV serotypes already early in life, starting around 2 years of age. Furthermore, the prevalence of antibodies to different AAV vector serotypes has been reported to vary according to geographical location [4, 5]. Therefore, it is of interest to determine the prevalence of NABs against different AAV serotypes in populations to be potentially treated with AAV-based gene therapy.

STUDY OBJECTIVE

Establishing anti-AAV NABs seroprevalence in a South African hemophilia B patient population (n=44) using a panel of AAV serotypes suitable for liver-targeted therapy. Results will determine the AAV serotypes likely to be of greatest clinical applicability for gene therapy in the South African hemophilia B population.

METHODS

Hemophilia B patient (n=44) and healthy donor (n=44) serum samples were obtained from a Hemophilia Comprehensive Care Center in Johannesburg (South Africa). All the serum samples were analyzed for the presence of NABs against AAV serotypes 1, 2, 5, 6 and 8 with the use of highly sensitive luciferase-based bioassays (Figure 1).

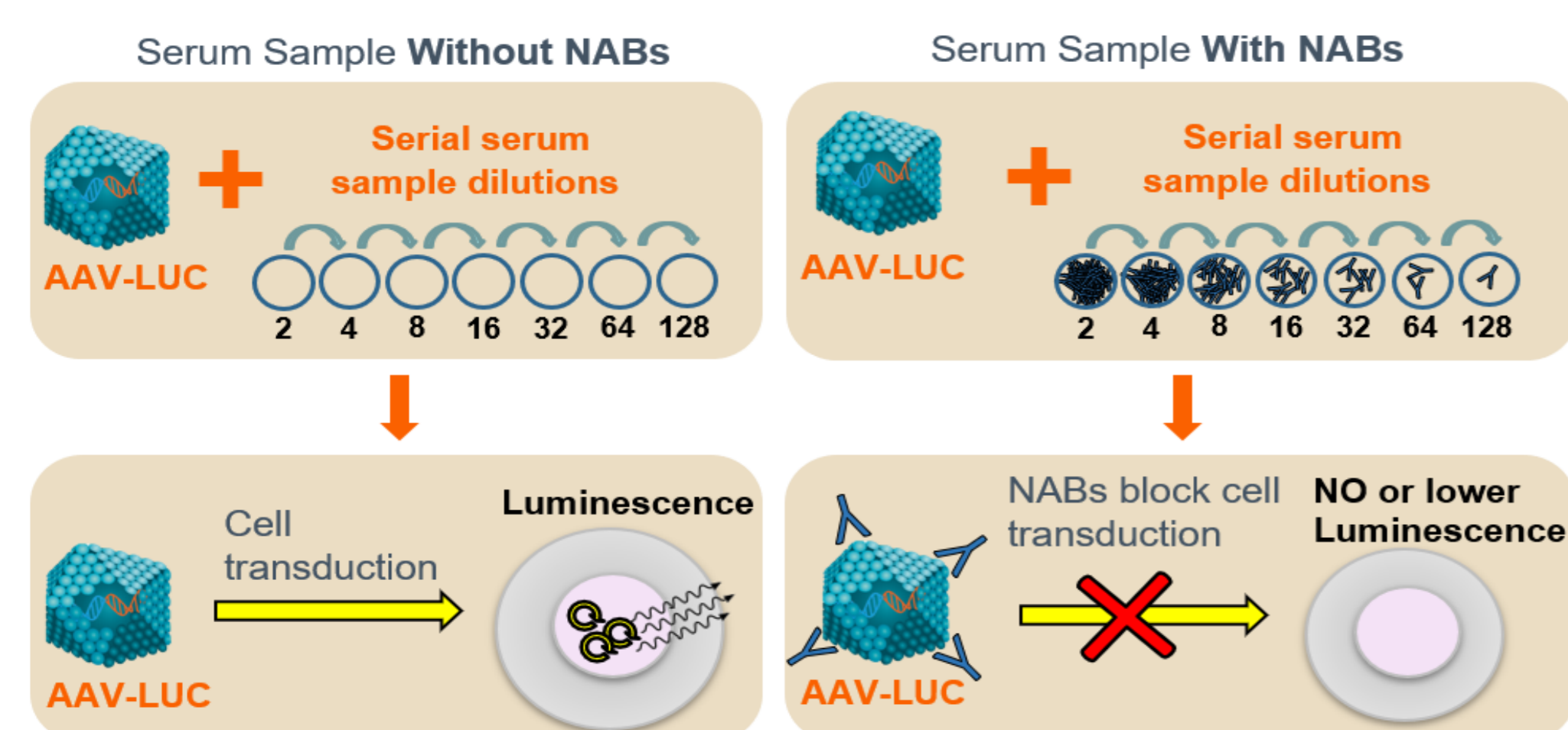


Figure 1. General principle of functional anti-AAV NABs detection.

Anti-AAV NABs titers were determined by calculation of the percentage of neutralization for each sample dilution and fitting the neutralization curve with a four-parameter method. Anti-AAV NABs titer (IC50) is the dilution at which antibodies inhibit Hek293T cell transduction with AAV-LUC by 50%. The lowest patient serum dilution used in every assay was 8. All analytical runs included proper negative and positive controls.

RESULTS

Anti-AAV5 and anti-AAV8 NABs are the least prevalent in both South African Hemophilia B patients and healthy donor population

- The presence of NABs against the AAV serotypes 1, 2, 5, 6 and 8 was determined in the serum of the hemophilia B patients (Figure 2, 3). The highest prevalence of NABs was found to be against the AAV2 serotype, 95% (n=42/44), followed by the AAV1 and AAV6 serotypes, both of which was 77% (n=34/44). The prevalence of NABs against AAV5 and AAV8 was the lowest, 64% (n=28/44) for both serotypes. Similar pattern was also observed in healthy donor population from South Africa (Figure 2).
- Serum samples positive for anti-AAV2 NABs had a highest occurrence of titers above 1030 (39%) in comparison to anti-AAV1 NABs (20%), anti-AAV5 NABs (5%) or anti-AAV8 NABs (7%). The occurrence of samples with low titers (ranging from titer of 9 to titer of 50) was the highest for anti-AAV8 NABs (32%) and for anti-AAV5 NABs (25%), followed by anti-AAV2 (18%) and anti-AAV1 (6%) (Figure 2).

Most South African Hemophilia B patients could benefit from AAV5 vector-based gene therapy

- Considering the anti-AAV NABs titer of 5 as being an exclusion criteria in most of the systemic AAV-based gene therapies, 23% of the analyzed patients could be treated with AAV1, 5% with AAV2, 34% with AAV5, 18% with AAV6 and 36% with AAV8-based therapeutics (Figure 4).
- However, we have previously shown that AAV5-NABs do not impair the efficacy of *in vivo* transduction of AAV5-based vector up to a measured titer of 340 in humans and 1030 in non-human primates [3].
- Applying the cut-off of 340 or 1030, either 84% or 95% of the South African Hemophilia B patients could benefit from treatment with AAV5-based gene therapy (Figure 4).

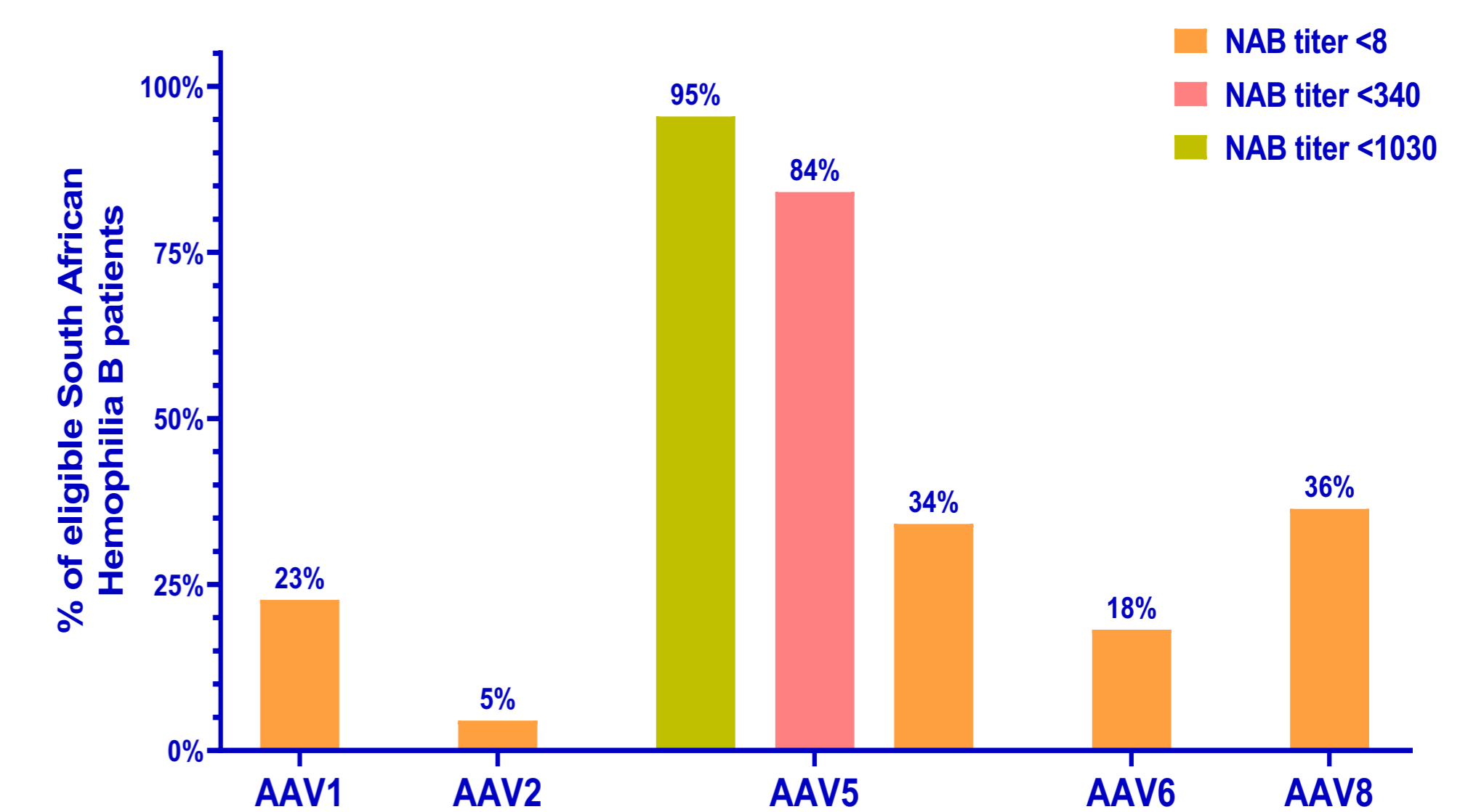


Figure 4 Theoretical eligibility of South African hemophilia B patients for systemic AAV1, 2, 5, 6 and 8-based gene therapy. Total number of analyzed patient serum samples was 44. NAB, neutralizing antibody; AAV, adeno-associated virus.

CONCLUSION

- Seroprevalence of pre-existing anti-AAV NABs among South African hemophilia B patients and healthy controls is considerably lower for AAV5 and AAV8 than for AAV1, 2, or 6.
- The results of our anti-AAV NABs seroprevalence study in a South African hemophilia B patient population show that at least 95% of those patients could benefit from treatment with AAV5 vector-based gene therapy, while 23%, 5%, 18%, 36% of the patients tested would be included in trials using systemic delivery of AAV1, AAV2, AAV6, or AAV8 vectors.

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DISCLOSURES

JNM has received research grants from uniQure, Bayer, Biogen, BioMarin, CSL Behring, Novo Nordisk, Sobi, and Roche. AM, FVV, SJVD, and VF are employees of uniQure N.V. Contact: Research and Development, Amsterdam, The Netherlands, a.majowicz@uniquire.com

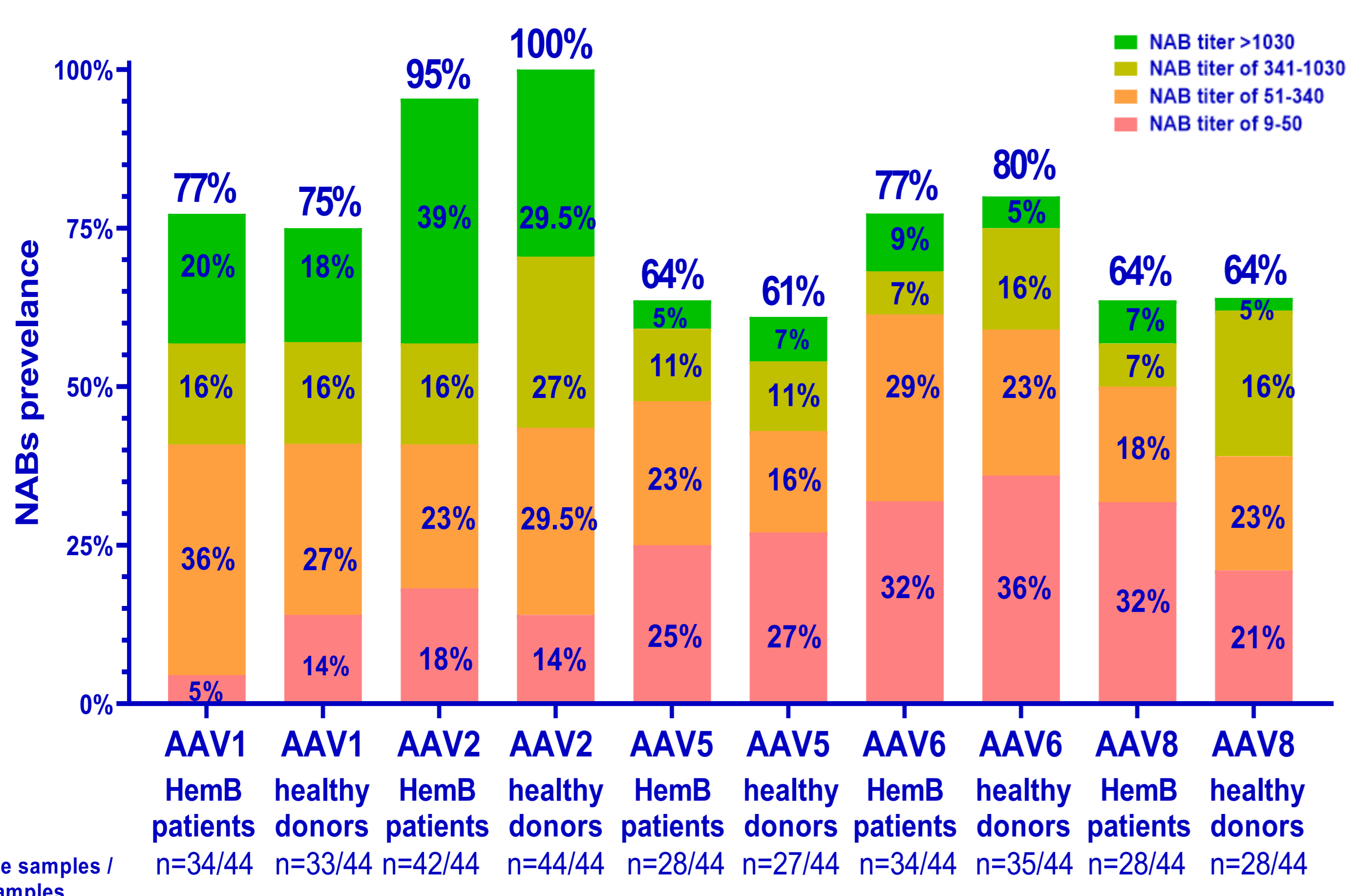


Figure 2. Prevalence and distribution of titers of pre-existing NABs against different AAV serotypes in South African hemophilia B patients and a healthy donor population. Total number of analyzed patient or healthy donor serum samples was 44 per group. NAB, neutralizing antibody; AAV, adeno-associated virus.

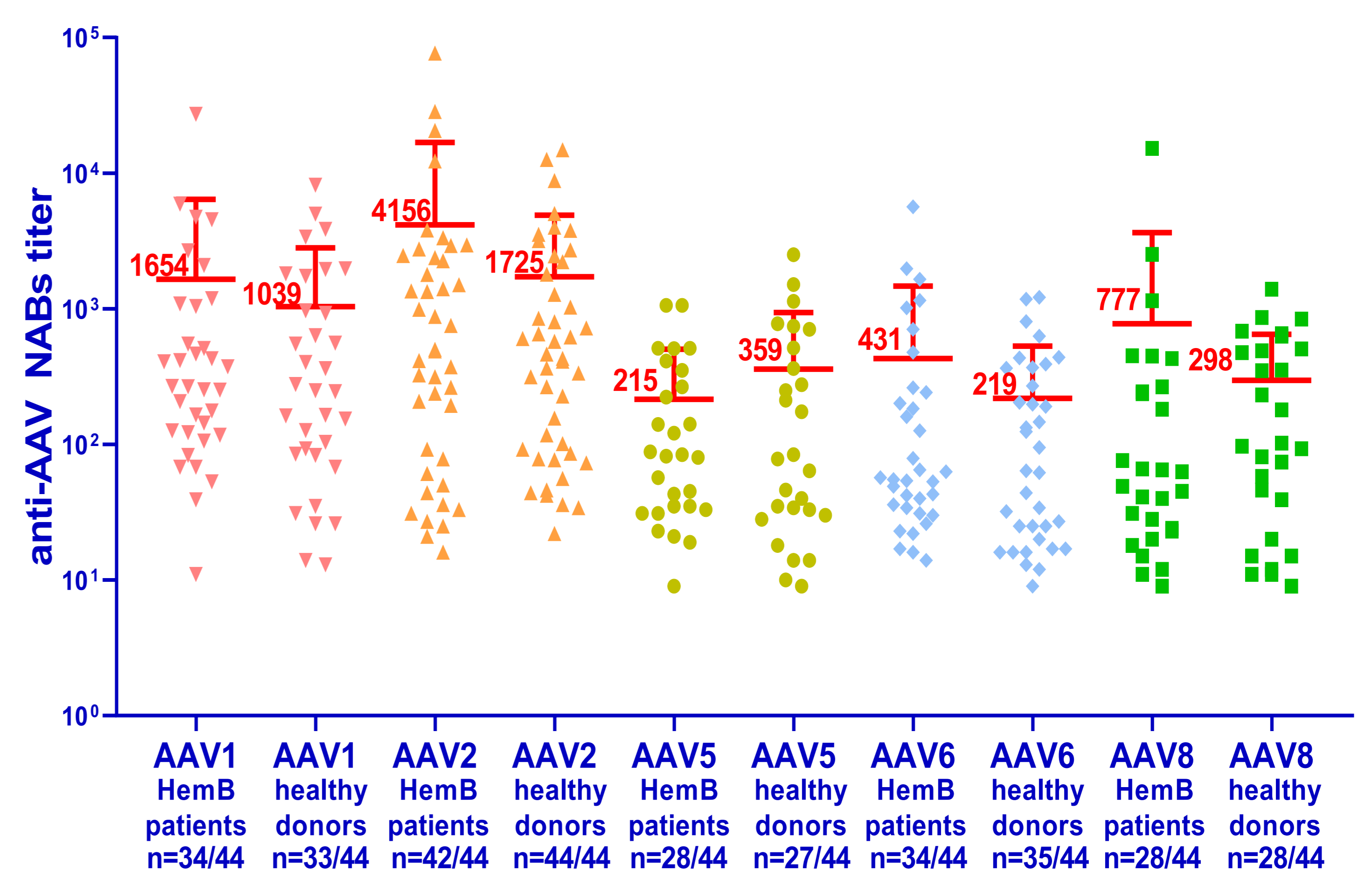


Figure 3. Individual anti-AAV NABs titers measured in serum of South African Hemophilia B patients and healthy donors. Mean \pm SD titer is depicted in red. Total number of analyzed patient or healthy donor serum samples was 44 per group. NAB, neutralizing antibody; AAV, adeno-associated virus; SD, standard deviation.